

Spectrographic Analysis and Studies on Lead Metabolism
with Special Reference to Industrial Lead Poisoning.

Thesis for the degree of Doctor of Medicine in the
University of Glasgow.

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PREFACE.

In presenting the following Spectrographic method of Lead analysis in biological material, and observations on Lead Metabolism I have set down the steps which I took as a newly installed Industrial Medical Officer in coping with several cases of alleged Industrial Lead Poisoning. While it was possible to obtain the bulk of the analytical material from the workmen under my care, I had to rely on the kindness of several of my colleagues to obtain samples for investigation of certain aspects of normal lead concentrations. I wish therefore to record my indebtedness to Dr. D. McKay Hart of the Maternity and Women's Hospital, Rotten Row, Dr. James Eaton of the Victoria Infirmary, and Dr. R. S. Barclay, Ruchill Hospital for their kindness and valuable co-operation in obtaining for me samples which it would otherwise have been impossible to procure. I am also deeply indebted to Professor Noah Morris for his criticism and advice as to the presentation of this thesis. To my good friend Mr. James McPheat, Chief Spectrographist of the Weir Metallurgical Laboratory who has taught me the art of accurate spectrography, and who has given me so much help in obtaining the reproductions of the spectrograms, I owe the thanks which are due his friendship and unrivalled spectrographic knowledge and experience. The work was carried out in the Weir Metallurgical Laboratory, and Medical Department, and to Sir John Richmond, and Mr. Malcolm L. Jamieson of the Board of Directors of G. & J. Weir Ltd., for their encouragement and permission to carry out the investigation, I also am indebted. The plates of the Spectrograph (p.13 and 15) and the Microphotometer (p.34 and 36) were obtained from blocks supplied by the courtesy of Messrs. Adam Hilger. With the exception of an article on the method and some of the results obtained, which has been accepted for publication by the Biochemical Journal, none of the work has hitherto been published.

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I N T R O D U C T I O N .

Poisoning by the heavy metals is of centuries old recognition, and descriptions of their toxic effects, from the writings of the early physicians has provided us with a group of toxaemias, which may be classed as the earliest Industrial Disease.

Of all the heavy metals, lead, by its ductility, ubiquity, and widespread use, and consequently prominent toxic effects, has claimed more attention than any of the others. Hippocrates in 370 B.C., described lead colic in the extractors and smelters of lead; to Nicander in the second century B.C., is attributed a description of lead poisoning, containing many of the cardinal symptoms and signs of modern clinical description. From these early physicians, we find that lead has attracted the attention of the profession throughout the centuries. In 1657 we find the well-known treatise of Stockhausen, and in 1703 (1) Ramazzini (2) included in his "Diseases of Tradesmen", accounts of Industrial Diseases, which today are known as Silicosis, Mercurial Poisoning, and Lead Poisoning. Probably the first classical description of lead poisoning is that of Tanquerel des Planches, (3) (1839),

and his work was the forerunner of the detailed investigation of Legge and Goadby, Oliver, Aub, Kehoe, and Hamilton, who attacked the problem from the biochemical side as an adjuvant to clinical diagnosis. We are forced to admit, however, that in spite of centuries old recognition, the toxic effects of lead are still a problem, and a source of professional controversy. In his book on Industrial Diseases, Johnstone would appear justified when he says, "It is a sad commentary on medical education that a disease known to the Ancient Greeks, Latins and Arabians, and studied by physicians throughout the centuries, inciting profuse material in medical literature, and having such well defined laboratory findings, should remain an enigma and source of controversy to the profession at large". On one point I disagree sharply with Johnstone; the laboratory findings are anything but well defined, and provide the main source of modern controversy, as a study of the literature proves.

This state of affairs is due to three main factors:-

- (1) Lead is protean in its toxic manifestations, and is to industrial disease, what syphilis is to

infectious disease.

(11) Lead is a normal constituent of body tissues, and fluids, and probably has a definite, but as yet undiscovered physiological function.

(111) The amounts of lead normally present in biological material are very small, and their estimation is laborious and difficult.

Wherever it be, the shade of Ramazinni must smile; his sword makers, and grinders of armour provided their quota of lead poisonings in the Middle Ages: today when armour is a strategic necessity, lead poisoning again looms prominently.

In the summer of 1941, a surprisingly large number of cases of colic, and gastro-enteritis, were encountered, mainly in the dilutee class of labour. Of these a few were diagnosed as lead poisoning outside the factory; within the factory, a few had to be considered as possible lead poisoning by reason of their pre-war employment, and the hazards of their war time occupation. In investigation of these cases as part of the Industrial Medical Service, several objections were found to the analytical methods in use. Of these, the two main objections were the operational time, and the amount of material required, and to these may be added the danger

of contamination error from the chemical reagents involved. It was accordingly decided to find a method which would be accurate, rapid, and require smaller quantities of material for analysis. In utilizing the Industrial Spectrograph for the quantitative analysis of lead in biological material, I believe that this purpose has been achieved, and the scope of this thesis will be to describe the elaboration of the spectrographic method, its advantages over the chemical method, and some observations on the biochemical behaviour of lead in the body. It will be obvious in passing that the use of the instrument holds promise of a rapid and accurate method of analysis for all the "trace" elements likely to be encountered in biological material.

SECTION I.

DISADVANTAGES OF CHEMICAL METHODS.

(10)

In 1838 Devergie and Hervey established the fact that lead is normally present in human blood, their work was later confirmed by Gautier, (1881).
(11)

These discoveries seem to have stimulated the minds of investigators, to devise methods of analysis for the qualitative and quantitative estimation of lead, no doubt as a basis for the diagnosis of poisoning where

abnormal concentrations were found. Progress seems to have been slow, however. Legge and Goadby ⁽¹²⁾ (1912), described a number of methods based on determination of lead as its sulphide, but admitted their limitations especially when applied to tissue; in view of modern knowledge, their methods would be untenable, especially from the criticism of contamination error and operational time. The modern approach to lead micro-analysis has been made from two sides; a colourimetric comparison estimation after extraction of the lead with chemical reagents on the one hand, or estimation of lead after its deposition after electrolysis; the lead is then extracted from the cathode and estimated by dithizone.

In the first group we find the original method of Fairhall ⁽¹³⁾ (1922), the S-Di-phenyl carbazide method which depends mainly on the estimation of lead in terms of the chromate after its conversion to lead chromate. Kehoe (1933), in his exhaustive study on lead metabolism used this method with modifications suggested by the work of Tannahill ⁽¹⁴⁾ (1929), and Francis ⁽¹⁵⁾ (1929). It is of interest to note that a method which had provided so many standard criteria in American Medical literature by its use in Kehoe's investigation should

have been subsequently abandoned by its originator.
(16) (17)
Fairhall, in reply to Kehoe's criticisms of some of Fairhall's results, pointed out that he had abandoned the method he had originally devised as it had been found inaccurate for quantities of lead of the order of one hundredth of a milligram. In spite of this it is clear that Kehoe's criticisms were justified. In turn Kehoe's claims as to the accuracy of the method he used are open to question mainly on the basis of contamination error, and occasional lack of sensitivity, which we now know probably originated in interference with the extraction of lead in the presence of bismuth, and/or tin. The method involved at least forty-three different chemical operations, and the use of twenty-three different chemical reagents. Obviously in view of the difficulty of obtaining lead-free reagents, there is here a source of contamination error, and also the risk of a considerable loss in the amount of the material to be analysed.

A distinct advance on this method was the
elaboration of the method of Tompsett and Anderson, (18)
(1935), which consists of three main steps; destruction of the organic material by ignition (in preference to digestion by sulphuric acid), extraction by Sodium-Di-Ethyl-Dithio-Carbamate of a lead-ether complex, and

estimation of the lead by dithizone. Interference with the extraction of the lead in the presence of iron or copper, are overcome by the addition of cyanide which also renders the dithizone solution stable. While there is still present a risk of contamination error, and loss of material in this method, the probability is much reduced by the reduction of reagents, and operational procedures required. Tompsett's results would seem to indicate that the method is highly accurate. Other workers, particularly in America, have devised refinements over the original Fairhall method; the scope for improvement is evident in the numerous improvements suggested by the same workers in successive publications. Bambach⁽¹⁹⁾ (1939), and Kozelka and Kluchesky⁽²⁰⁾ (1941), have devised improvements directed mainly to the overcoming of the interference to the extraction of lead, in the presence of tin and bismuth.

Cooksey and Walton,⁽²¹⁾ (1929), devised an electrolytic extraction of lead after precipitation from solution; and a similar method was published⁽²²⁾ about the same time by Francis, Harvey, and Buchan, (1929). In their application of the method to the analysis of

urine, they experienced the same difficulty as I myself encountered in an attempt to obtain lead-free standard material for urinary analysis; namely, that electrolytic deposition of lead on the cathode is incomplete, and full extraction of the lead is never attained. Further, the necessity of first precipitating the lead from solution leads to loss of material.

(23)

Bambach and Cholak, (1941), would seem to have overcome this difficulty by direct electrolysis, and extraction of the lead from the cathode by dithizone; the main conditions for the successful application of this method, are maintenance of the electrolyte at pH.3 to 4, to prevent precipitation of lead and calcium phosphates, and also the overcoming of interference to lead deposition in the presence of iron and phosphates. A further step from the electrolytic method is the use of the dropping mercury cathode in the polarographic method of Cholak and Bambach, (1941), (24) but it is presented more for its academic interest than practical application. Comparison of results by this method indicate that it is comparable in its accuracy, with the Spectrographic, and best chemical methods. Of these two main methods of analysis, (chemical and electrolytic), the chemical method would seem to be preferable. The liability to erroneous results due to contamination error and loss of material is however

always present, further the operational time and the quantity of analytical material required are so considerable that chemical methods are impracticable for many of the purposes to which it is desired to apply them. Kehoe in his investigation used fifty cubic centimetres of blood, and required an operational time of forty-eight hours, stressing the necessity of constant supervision. Obviously the method does not permit of repeated examination, is inapplicable to the analysis of serum, and cerebro-spinal fluid, and is a laborious and slow procedure.

Tompsett and Anderson's method is considerably wider in its application, and faster, requiring twenty cubic centimetres of blood, and an operational time of thirty-six hours; it is of doubtful application to cerebro-spinal fluid analysis, and again only one sample can be estimated at a time, with the additional labour of blank estimations to check accuracy.

Viewing Industrial Spectrographers at work, one cannot fail to be impressed by the speed and accuracy with which a large number of samples can be analysed, where the amount of analytical material at their disposal is very small. Application of the spectrographic method to the analysis of lead in biological material eliminates the objections outlined above, with the outstanding

advantages of speed of working by reason of the short operational time required, the reduction of chemical manipulations to a minimum, and the small amounts of material required for analysis.

SECTION II.

HISTORY OF THE SPECTROGRAPH AND METHOD OF USE.

In 1672 the discovery of the dispersion of light was made by Newton, ⁽²⁵⁾ but it was not until 1817 that ⁽²⁶⁾ Fraunhofer constructed the first spectroscope. He used this instrument, among other things, for the observation of metallic emission lines, (the sodium doublet in the yellow), and the spectrum of the electric spark. Several years elapsed before it was realized that the lines in the yellow part of the spectrum observed by Fraunhofer were due to metallic emission. The first realization of their metallic origin is credited to ⁽²⁷⁾ W.H. Fox Talbot, (1836). Further advances in observations on the spark spectra of other metals were ⁽²⁸⁾ made by Wheatstone (1835), and ⁽²⁹⁾ Crookes, (1856), who photographed the spectrum in the ultra violet region, using to all intents and purposes, what was the first quartz ⁽³⁰⁾ spectrograph. Kirchoff and Bunsen (1860), made discoveries of great importance, and comparable with those of Newton and Fraunhofer. They definitely proved that the flame

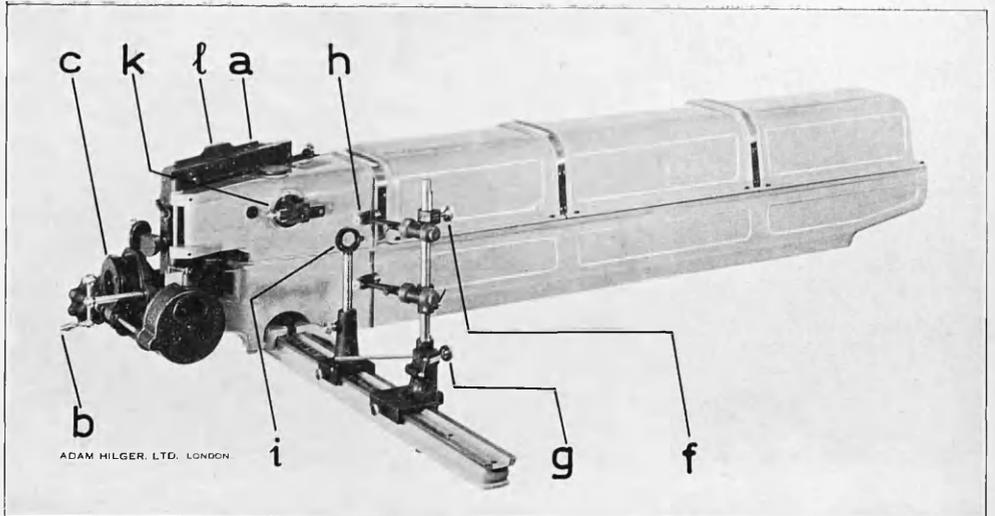
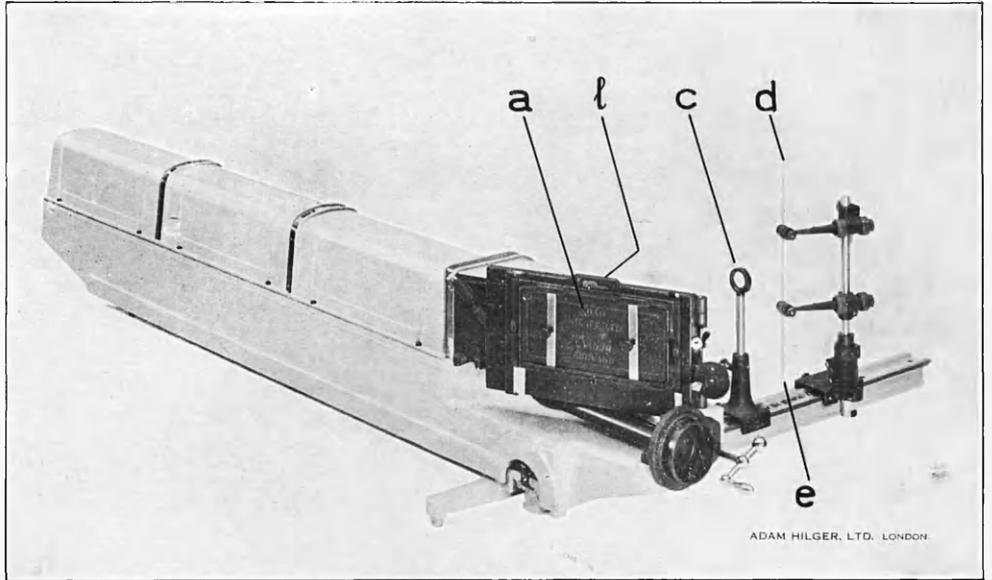
spectra of metals were almost identical with the spark spectrum between electrodes of the metal, and that the same metals always produce the same lines, independent of the substances with which they are combined, (e.g., magnesium would emit the same lines as magnesium sulphate, magnesium carbonate, etc.) They further concluded that certain lines on the solar spectrum coinciding with the spectrum lines of certain metals, indicated that these metals were constituents of the sun. This led them to suggest that spectrum analysis provided a very sensitive method of estimation for these substances.

Another aspect of spectroscopy to which he gives his name, is found in the work of Angstrom, (1869).⁽³¹⁾ Elaborating on the work of Kirchoff and Bunsen, he proved the existence in the Sun and Earth of the same elements, and devised a unit of measurement by which each element could be identified in terms of wavelength. These units were expressed in ten millionths of a millimetre, and are known as Angstrom units, denoted by the letter A^o.⁽³²⁾ Lockyer, (1874) took definite steps for the elaboration of quantitative spectroscopic methods,⁽³³⁾ and these were continued by Hartley (1883).

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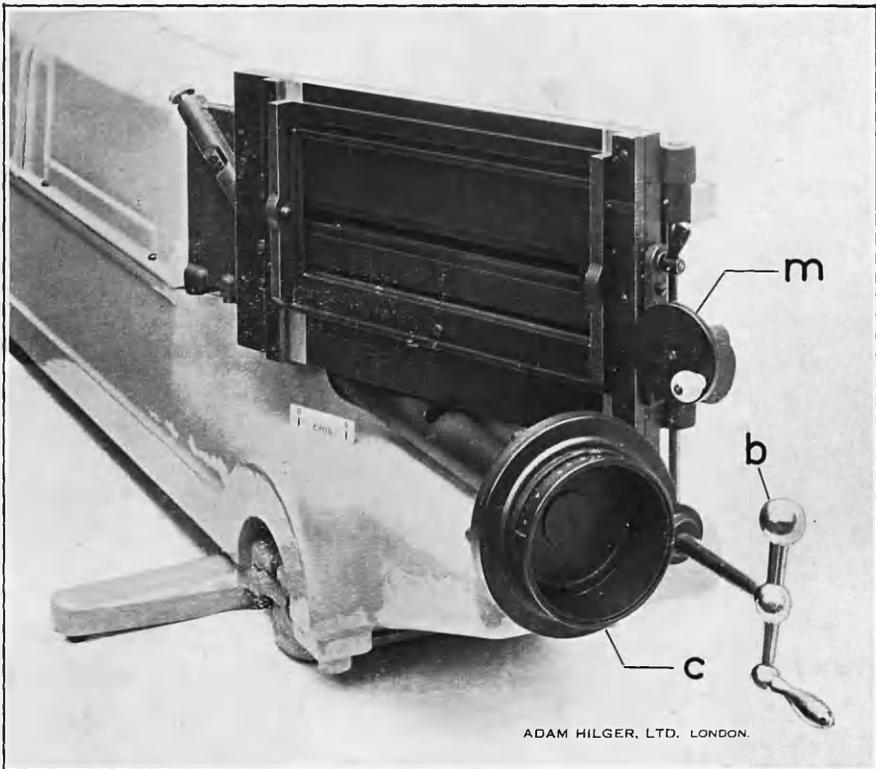
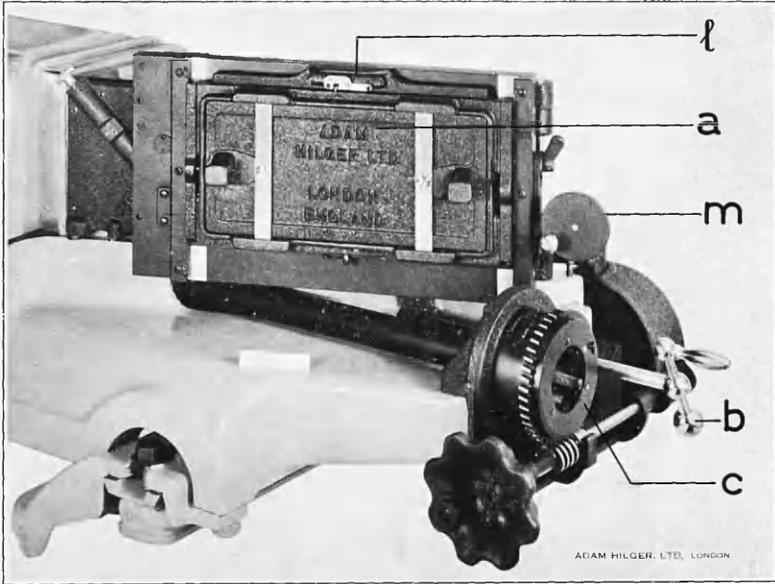
The discovery by De Gramont, (1907), that certain lines emitted by elements are indicative of the presence of that element in minimal concentrations, was a further contribution of great practical value, particularly to biochemical analysis where the concentrations dealt with are so weak. In a concentration of, say, one part per million, an element might only emit one line, whereas in a concentration of two parts per million it might emit four. To those lines which persist in diminishing concentrations, De Gramont gave the appellation "Les Raies sensibles;" to the lines to disappear last, the designation, "Les raies ultimes"; these lines on spectrograph wavelength charts carry the notation "a", and the less sensitive lines are notated "b".

The discoveries by these workers, plus modern advances in optical science, has led to the evolution of the modern spectrograph, which industrially has found such a wide field of application in the hands of such workers as Twyman, (35) Barker (36) and McPheat. (37) It embodies among others these outstanding advances over older methods; the use of a collimating lens to maintain the lines in a dispersed measurable state, and the permanent recording of the emission spectra on

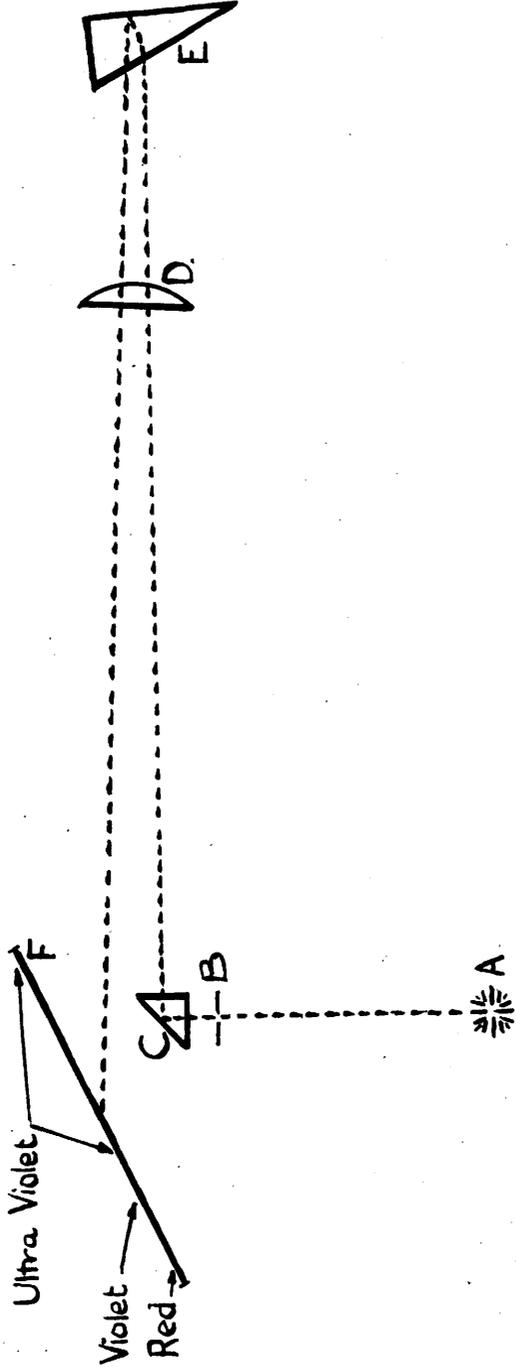


a photographic plate; it further facilitates rapid and accurate analysis, by providing standard conditions of current, illumination, and exposure. The instrument used is the large Hilger spectrograph, and bearing in mind the initial objects, viz., to bring the metallic elements into a state of atomic excitation, and to direct the rays of light emitted by the vaporised metal on to the photographic plate, the procedure is as follows.

The plate holder (a) is loaded with the plate, and fitted to the rack, where it is held in position by a simple locking device. The wavelength band is adjusted by means of a rotating wheel (b), the wavelengths it is desired to use for analysis being set from readings marked on the drum (c). The current and resistance are set by a simple plug-in arrangement, and resistance box. The electrodes (d. and e.) are held by means of copper vices so constructed, that they permit of adjustment of the electrodes without the arc points being contaminated by contact with the metal. The electrodes can be raised and lowered up and down the vertical stand by means of screw wheels, (f. and g.) The whole apparatus for holding and adjusting the electrodes is known as the "Gramont stand", after its originator. The adjustment is such that the electrodes are focussed on a fixed point



on the target (h) when viewed through the magnifying offset lens (i). This is necessary to ensure that the maximum illumination from the electrodes will always pass through the spectrograph slit (k). The plate diaphragm (l) is raised exposing the plate, the timing clock switched on, and the arc excited. The sample burns with an incandescent flame in which the colours, typical of some metals, may be observed. Inspection of the image of the flame, playing around the target spot, indicates as to whether the setting of the electrodes is satisfactory or not. The train of events will be better understood by reference to figure (3). The light from the sample (A) passes through the slit of the spectrograph (B) and the right-angled prism (C). The rays of light, after passage through this right-angled prism, pass through a collimating lens (D), and then through a 30° Littrow prism. (E) This prism splits up the composite light into its constituent rays, which, reflected back from the second surface of the prism, pass once more in their dispersed state through the collimating lens (D), and thence to the photographic plate; in this way the composite light emitted from the sample, on its atomic excitation by the current, is photographed on

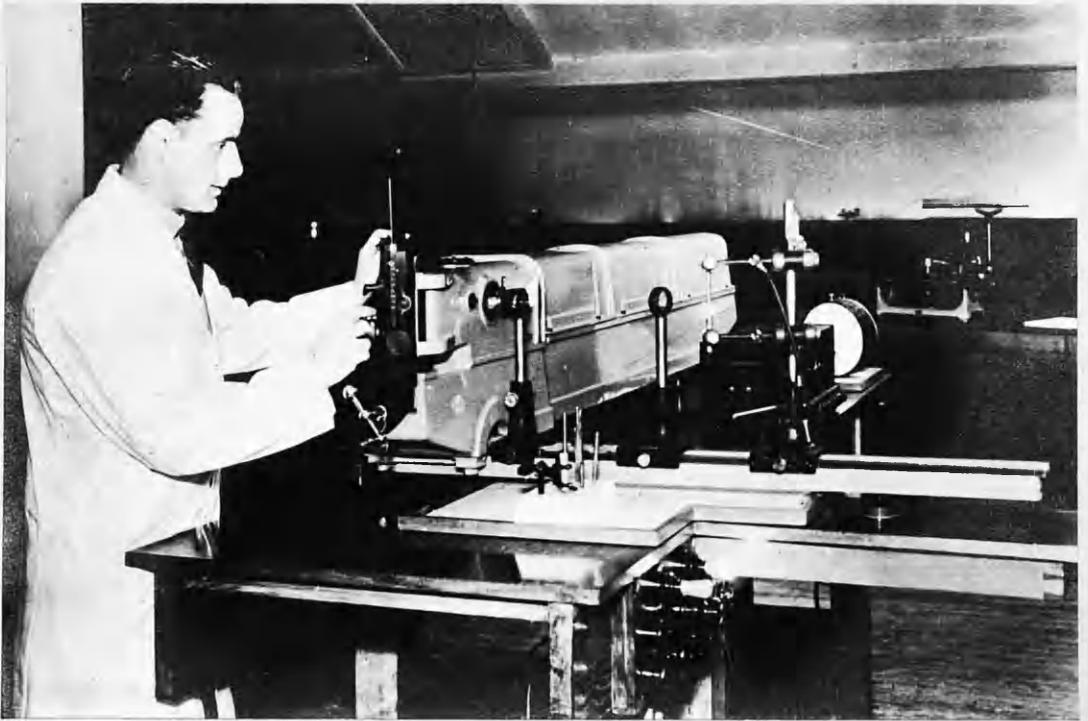


— LARGE QUARTZ SPECTROGRAPH. —

— OPTICAL TRAIN. —

the plate in terms of its constituent rays. The purpose of making the rays of light thus travel twice the length of the spectrograph and then through the collimating lens is in order to gain as much dispersion as possible; in this way better resolution of the emission spectra is obtained on the photographic plate. The prism and lens are made of quartz, to permit of transmission of ultra-violet light as it is in the ultra-violet region that the majority of metallic emission spectra are found. The rotating wheel (B. fig.1) acts by altering the angle at which the optical elements are set, and in effect is such that the region of the spectrum field not under examination is made to miss the action of the collimating lens and prism, so that the emission spectrum in that particular wavelength never reaches the photographic plate. The plate is raised after exposure is complete, by means of the rack handle (m), a convenient distance is three millimetres, and the next sample can now be exposed. In this way as many as twenty samples can be photographed on the same plate. The plate diaphragm is now closed, the plate removed, developed, dried and viewed.

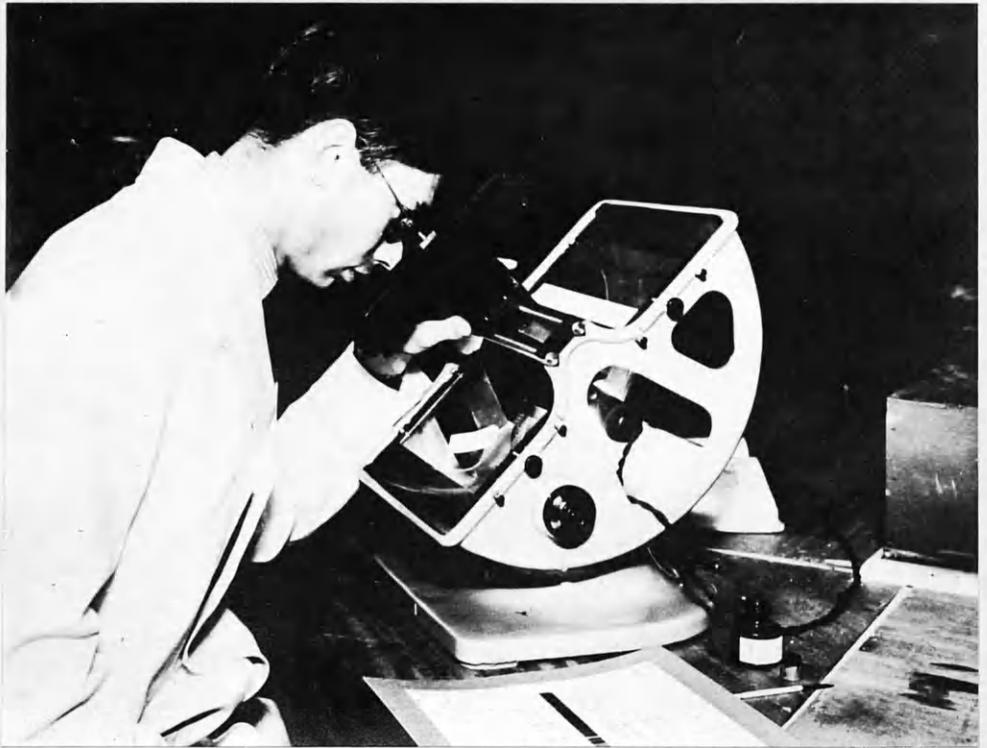
The viewing is best accomplished by means of a special piece of apparatus, the Judd Lewis Comparator,



The sample is seen being arced and emitting light between the electrodes. The plate diaphragm is in the raised position exposing the plate upon which is photographed the rays of light once they have passed through the optical elements embodied in the spectrograph. The operator is seen ready to raise or lower the plate by means of the rack-handle. It is not shown in the photograph, but in the Weir Laboratories a screen capable of cutting out ultra-violet light is attached to the "Gramont" stand, enabling the operator to view the arcing of the sample without danger to his eyes from ultra-violet radiation.

which permits of simultaneous microscopic examination of two plates at four magnifications. It is seen that there are several hundred dark lines on the plate, each occupying its own place; since the emission spectrum of an element is of a wavelength specific to itself, it follows that no two elements can occupy exactly the same site on the spectrum field, (Kirchoff, Angstrom, etc.) It must be pointed out that some elements are capable of emitting several hundred lines (Iron), others emit comparatively few (Gold, Thorium and Arsenic.) If however iron were present in a weak concentration or entirely absent, the number of lines would be considerably reduced, or in the latter case absent altogether leaving a blank space on the photographic plate, - proof of the truth of Kirchoff and Angstrom's findings. Identification of the element of origin of the lines is achieved by aligning the test plate with a standard plate on which the elements are known and marked. The standard plate is placed on the upper stage, the test plate on the lower stage; these stages are movable within small limits by means of the screws at the side of the stage. Certain lines by their prominence are at once easily recognizable, particularly if they

JUDD LEWIS COMPARATOR.



The operator has aligned the standard plate on the upper stage, and the test plate on the lower stage, by means of the adjustments which are seen at the side of the stages. The microscopic adjustment through which he is looking is movable along the whole length of the plate. On examination the lines are seen as shown on the spectrographic plates.

exhibit the phenomenon of doublets, i.e., two lines of the same element side by side, or triplets (Iron 3,100). These prominent lines are aligned on each plate, forming fixed points between which all other lines which align on the two plates are necessarily of the same element. Thus if after aligning the iron, magnesium and copper lines on the standard and test plates, a line on the test plate coincides with lead, tin or silicon lines on the standard plate, it indicates the presence of these elements in the sample analysed; this necessarily follows since the emission spectrum of an element, is of a wavelength specific to itself. The lines are marked with their appropriate chemical symbol, and their concentration in the sample can be measured by suitable means.

SECTION III.QUALITATIVE SPECTROGRAPHIC ANALYSIS OF BLOOD FOR LEAD.

The arm of a selected subject was washed with lead-free alcohol, and two blood samples of five cubic centimetres drawn from the arm (samples A. and B.), by means of a Pyrex syringe fitted with a stainless steel needle. The samples were immediately transferred to a lead-free silica vitreosil crucible. To sample B. was added ten microgrammes of pure metallic lead in solution. The samples were then sulphated by the addition of three cubic centimetres of concentrated sulphuric acid which had previously been determined to be lead free. This conversion of the metallic elements present in the sample to the sulphates renders them stable. The samples were next placed in a dust-proof electric muffle, and maintained at a temperature of one hundred and ten degrees centigrade for twenty-four hours. This achieves a reduction in volume preparatory to elevation of the temperature to five hundred degrees centigrade, exposure to the latter temperature for twelve hours sufficing to ash the samples. The ash so obtained was a grey decrepitated mass with a rusty tinge throughout. The ashes were ground to powder in a clean agate mortar by means of an agate pestle. The ashes were then weighed, and

BLOOD SPECTROGRAMS IN THE REGION 2,800/3,000 A⁰.



When viewed through the Judd Lewis Comparator, the developed plate presents the appearance shown above. The photograph is of two identical blood samples, arced under widely different conditions of exposure. This is well shown by the very marked increase in density of the magnesium line 2852 A⁰, and shows the necessity of maintenance of standard conditions in exposure and current. The magnesium line in spectrogram 2 is so dense that it would probably be impossible of measurement. The lead lines are well shown. The line adjacent to Pb 2833 A⁰ is an iron line.

spectrographed under the following standard spectrographic conditions.

Instrument. Large Hilger quartz spectrograph.

Waveband. 2,700 A°.-- 4,300 A°.

Electrodes. Upper positive:- H. and D. graphite electrode 6.5 millimetres in diameter pointed to 80° angulation.

Lower negative:- H. and D. graphite electrode 10 millimetres diameter cut in the shape of a crater to receive the ash.

Arc Gap. 2 Millimetres.

Current. 110 volts D.C. 5 amperes.

Width of slit. .015 millimetres.

Exposure. 40 Seconds.

Plate. Zenith Kodak plate. H. and D. 700.

Developer. Standard spectrograph developer for 90 seconds at 67° F.

Fixer. Fix in acid hypo for fifteen minutes,
wash in running water for fifteen minutes.
Dry in a current of warm air.

In blood the following lead lines are easily recognizable at 2823 A°, 2833 A°, 2873 A°. The sensitivity of the method was at once apparent in the

lead line of sample B. This result was confirmatory
of the work of Shipley (1932)⁽³⁸⁾, and it was at once
obvious that the method would lend itself readily
to quantitative analysis by reason of its sensitivity.
The lead line at 2833 A⁰ was selected as the line
for working, because its proximity to the heavy
magnesium line (2853 A⁰) rendered its recognition
easy. It was accordingly decided to elaborate a
quantitative spectrographic method for the analysis
of lead in biological material.

SECTION IV.

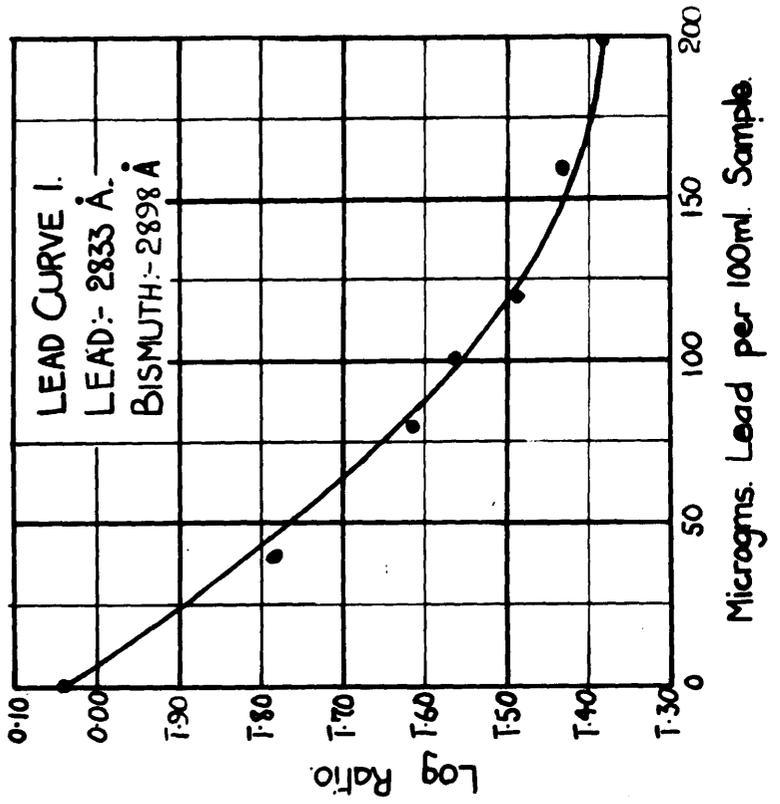
QUANTITATIVE SPECTROGRAPHIC ANALYSIS OF LEAD IN BLOOD.

In considering the elaboration of a quantitative
method several possibilities presented themselves.
It would have been possible to proceed along the lines
of Sheldon (1931)⁽³⁹⁾, and compare the unknown samples
with those of standard samples in which the lead content
is known, by a visual comparison of line densities.
One obvious objection to this is that there is no
guarantee that the samples will emit the same illumination
through the slit; in fact it is easy to visualize
circumstances in which inequality of illumination would
be present; this invalidates comparison with standard
solutions. Sheldon, himself, drew attention to the
inaccuracy, and lack of sensitivity of certain aspects

of the method he employed. Use of flame spectra such as he used is the least sensitive spectrographic method, and in his work, while still demonstrating the possibilities of the method, the lack of sensitivity probably originated from incomplete combustion of material. Industrially the method in use for years has been the method of Internal Reference, for which much of the credit is given to Gerlach, (1925).⁽⁴⁰⁾

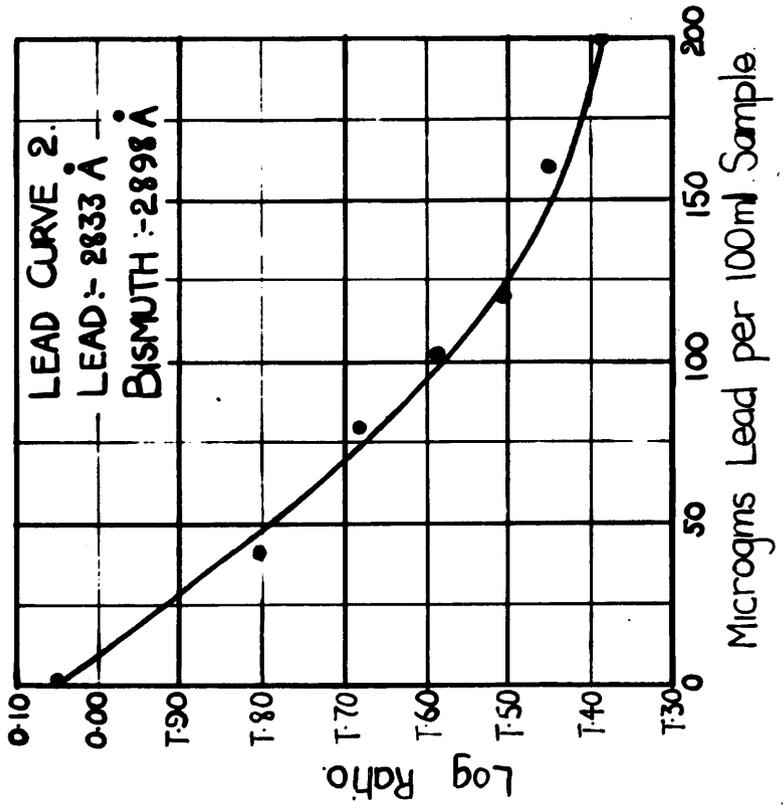
This method is dependent on the fact that the line densities of the emission spectra of elements as measured by the microphotometer are directly proportional to their molecular concentration in a given sample when spectrographed under standard conditions of current, exposure, and development.

It is thus possible to plot a graph of the ratio of the line densities of varying quantities of lead and a constant amount of an internal reference element which is always added to the sample before it is spectrographed. All that is necessary once such a standard graph has been plotted, is to measure the densities of a lead line (2833 \AA^0) and that of the chosen internal reference element, and read the quantity of lead present from the ratio of line densities on the graph. In adopting the method of Internal Reference these problems at once presented themselves.



- (I) The selection of a suitable internal reference element.
- (II) Preparation of standard material from which to plot the graph.
- (III) Introduction of the internal reference element to the sample.

After some consideration it was decided to use bismuth as the internal reference element and the bismuth line at 2898 \AA° as the internal reference line. At the time bismuth had never been detected in blood, and it could be easily introduced to the sample in the sulphuric acid used for sulphation. The only criticism appeared to be that the distance between the lead line (2833 \AA° .) and the bismuth line (2898 \AA° .) was rather wide for accurate measurement. For the preparation of the graph it would have been possible to use samples of blood, to which could be added lead and bismuth, but this would have entailed measurement by chemical methods of the lead already present in the blood, and involved the use of large quantities of analytical material. The metals present in blood are fairly constant, although varying in state of combination and concentration. Since, however, it is only the main base spectrum that is required, it was considered



feasible to prepare the standards from a synthetic lead-free blood ash. This synthetic blood ash was prepared in the following approximate proportions from pure Analar Carbonates, except where the metals could be obtained as the sulphates, (as this is the form pertaining after sulphation).

Sodium Sulphate (anhydrous) 617.7 grams.

Potassium Sulphate (anhydrous) 445.6 grams.

Calcium Sulphate (anhydrous) 20.38 grams.

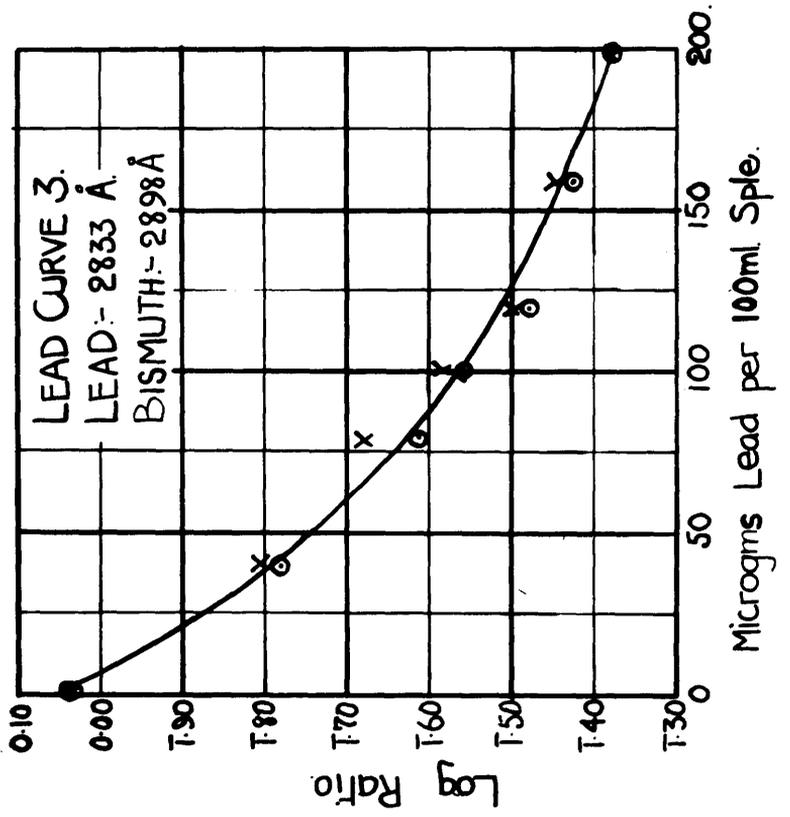
Magnesium Sulphate (7 H₂O) 30.36 grams.

Ferrous Ammonium Sulphate (6 H₂O) 351.1 grams.

Zinc Sulphate. (7 H₂O) .2904 grams.

(Copper Sulphate no data.)

The synthetic ash so procured forms a chalky white powder, rendered lead-free with considerable difficulty, and only after prolonged refining. All the supposedly pure chemical reagents contained traces of lead, and the ash ultimately obtained probably did contain minute quantities of lead, (1 - 2 microgrammes per cent), even after attempts at purification by electrolysis. Five cubic centimetres of blood when sulphated and ashed under the conditions stated, weighs from .05 grams to .06 grams. The former quantity of synthetic ash was therefore used for the



addition of bismuth of which 5.6 microgrammes were added in 3 cubic centimetres of lead-free concentrated sulphuric acid. The quantity of bismuth added was governed by the attempt to obtain a graph with a straight line function, but this could not be achieved; the lead additions are shown below.

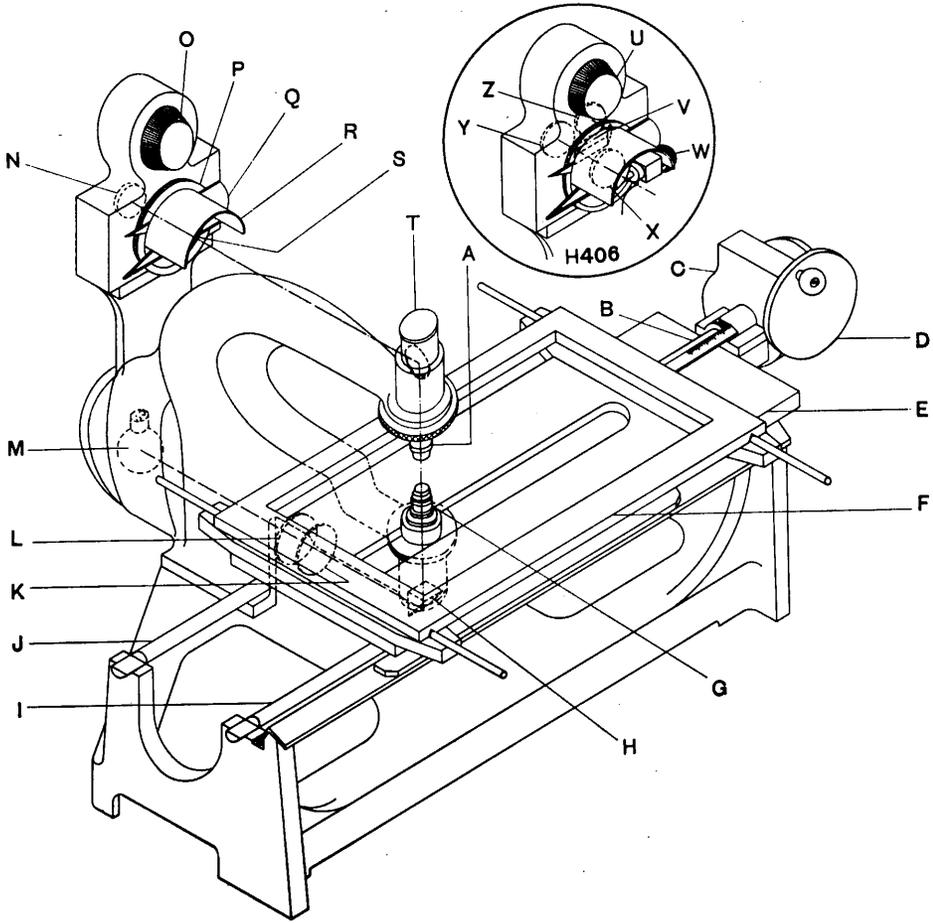
TABLE I.

<u>Sample.</u>	<u>Lead solution added c.cs.</u>	<u>Equivalent to Lead addition in microgrammes per 100 c.cs. of blood.</u>
1	Nil	Nil
2	0.2	40
3	0.4	80
4	0.5	100
5	0.6	120
6	0.8	160
7	1.0	200

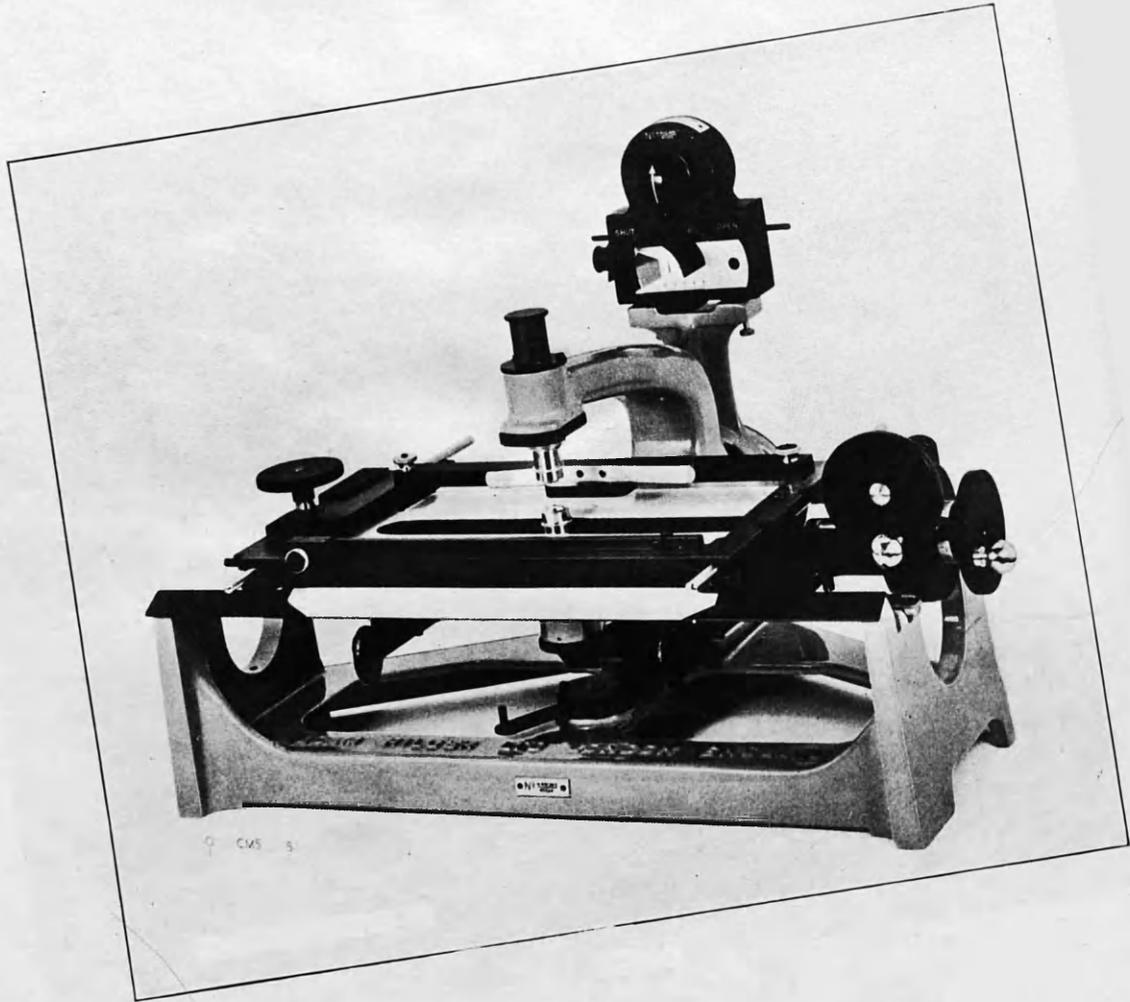
Two sets of samples were spectrographed, the points cross-checked, and the mean of the two results taken as the standard (Lead curve 3).

The lines once identified on the Judd Lewis Comparator were marked with a small dot, red (lead) and green (bismuth), and the line densities measured by means of the Hilger non-recording microphotometer.

This instrument consists of a light source (M)



so arranged that the beam of light emitted, in passing through the objectives (G. & A.) by an arrangement of prisms and lenses, is directed through the photo cell slit (S) behind which is housed a small photo-electric cell (N). Between the two objectives (G. and A.) is a sliding stage (F) controlled by fine and coarse adjustments (D. and C). This stage holds the developed plate and by means of its adjustments the lines on the plate are made to traverse at right angles the beam of light between the objectives (G. and A). The jaws of the slit are so treated that they form a screen on which the image of the lines marked as described are viewed at ten magnifications lying parallel to the slit, across which they can be moved by means of the fine adjustment to the sliding stage (F). The instrument is fitted with a variable shunt (O), and the slit is protected from the intrusion of extraneous light by a small hood, (R). Readings are made on the scale (Q), marked in centimetres. The photo-electric cell is connected with a galvanometer (X), the image of the galvanometer deflections being reflected by the galvanometer mirror on to the scale (Q). By means of the variable shunt, the image of the galvanometer deflection on the scale is always set at thirty centimetres for lead investigations, although



I have since observed that it makes little or no difference if the setting is raised to fifty centimetres. This I have had to do on occasions when the lead content of the sample was beyond the range I had calculated as maximum. Once the shunt setting is made, anything which reduces the intensity of the light passing through the slit will reduce the excitation of the photo-electric cell; this reduced photo-electric response is at once evident in a drop of the galvanometer reading on the scale. On manipulation of the fine adjustment (D), the interference to the beam of light by the lead and bismuth lines on traversing the slit, causes such a drop in the galvanometer reading on the scale. Since the density of these lines is in direct ratio to the molecular concentration of lead and bismuth in the sample, the galvanometer deflections as read on the scale provide a means of their measurement. It is more convenient to use the logarithmic ratios of such readings, rather than the direct ratios, as the graphs so obtained approximate more closely to a straight line.

33 28

OPERATION OF THE MICROPHOTOMETER.



The operator by means of the fine adjustment is bringing the line under examination across the photocell slit. The photograph does not show the galvanometer. The light reflected from the galvanometer mirror is seen as the small bright disc on the scale. The shunt setting is thirty centimetres, and once the line occupies the photocell slit, interference to the passage of light through it is shown by a movement of the galvanometer reflection to the left, which varies in accordance with the density of the line under examination.

This probably originates in the fact that on exposure, the response to light of the photographic emulsion to produce darkening of the plate, is logarithmic in character over a given period. In using the microphotometer it is essential to see that the image of the lines are in exact focus, that they are lying parallel to the slit, and that they occupy the whole slit.

The next step was checking the accuracy of the graph. This was done as follows. Several pairs of identical samples of blood and synthetic ash were used. To one sample unknown to the operator a lead addition was made and he was asked to state the amount added. The additions and recovery are shown below:-

TABLE II.

Sample.	Ash Wt.	Add.	Result.	Error.
		Microgms/ 100 mls.	Microgms/ 100 mls.	Microgms/ 100 mls.
(A) 5 mls. whole blood.	.0506 gms.	-	18	-
(A2) 5 " whole blood. and lead.	.0522	" 40	60	+2
(B) Whole blood.	.0510	" -	17	-
(B2) Whole blood and lead.	.0526	" 80	100	+3
- Standard ash 1+lead.	.0500	" 50	48	-2
" " 2+ "	.0500	" 100	108	+8
" " 3+ "	.0500	" 120	116	-4
" " 4+ "	.0500	" 140	138	-2

The slight errors shown above are of no significance being of the order of .000002 to .000008 grams. per 100 c.c; applied clinically, they cannot affect a diagnosis or obscure it.

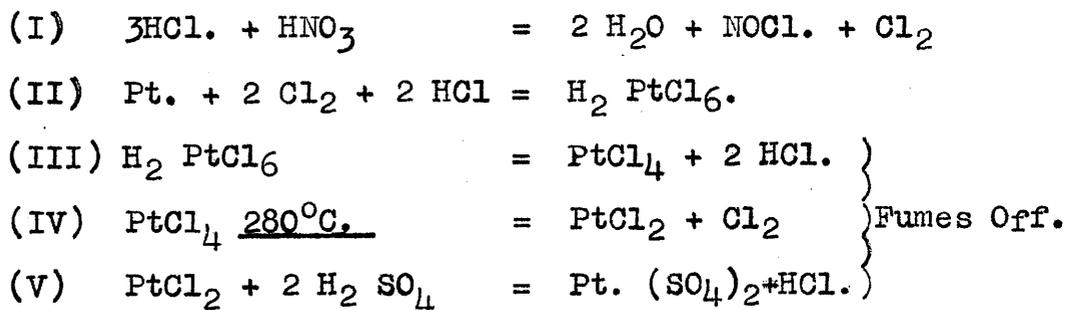
The lead curve 3 was used on several samples collected from volunteers, and suspect cases of lead absorption, with results which indicated that the method was highly accurate. When a sufficient volume of blood can be submitted to give two equal samples, the accuracy of the result obtained, can be checked by the method of blank additions outlined above. It seemed that a highly accurate method of analysis for lead concentrations in blood had been elaborated. As was inevitable, however, bizarre results were encountered in odd cases, which led us to suspect a source of base error. With greater experience I am now aware that these results were accurate but at the time several results, particularly those which showed low concentrations raised doubts.

One of the cardinal sources of error which had always to be reckoned on as a possibility was the chance that bismuth would be present in the sample prior to the bismuth addition on sulphation - the more so, as it is a favourite constituent of the therapeutic measures in vogue for gastro-intestinal disturbance, a syndrome which is an invariable accompaniment of heavy metal

poisoning. A series of samples from individuals exposed to bismuth absorption therapeutically did indicate the presence of bismuth in the circulation by a definite line at 2898 A° . Shortly afterwards a sample of blood submitted from a large city hospital showed a strong bismuth line in the field of election. (Page 47. 4). The effect of bismuth when present normally is that the bismuth line at 2898 A° is intensified, and on comparison with the lead line leads to an erroneously low result. What element, therefore, was to be used in place of bismuth, with the main qualifications, that it would never be found in body tissues or fluids, would give a measurable line reasonably close to the lead line at 2833 A° , and would not be overshadowed by any of the blood element lines. The choice fell on platinum which on spectrograph charts emits a line at 2830 A° , and fulfilled the considerations outlined above, while being in a position of proximity to the lead line at 2833 A° , ideal for measurement. The platinum solution for addition to the blood samples was prepared as follows:-

.5 grams of pure platinum were dissolved in the minimum amount of pure Aqua Regia, and 10 cubic centimetres of concentrated sulphuric acid (lead-free) added. The mixture was then taken down to copious fumes

of SO_3 on a hot plate, cooled and brought up to five hundred cubic centimetres volume with pure concentrated sulphuric acid. The platinum dissolved in Aqua Regia forms a solution of Chloro-Platinic Acid ($\text{H}_2 \text{PtCl}_6$). After the addition of Sulphuric Acid and the application of heat, the Hydrochloric and Nitric Acids fume off between 120°C . and 170°C .. At this point, the Chloro-Platinic Acid decomposes, and gives temporarily a reddish-yellow precipitate of Platinum Tetrachloride. When the Sulphuric Acid commences to fume in the region of 370°C . the Platinum Tetrachloride decomposes further into Platinum Chloride and Chlorine, the latter fuming off at the high temperature leaving a solution of platinum sulphate in concentrated sulphuric acid thus:-



Fifty cubic centimetres of this solution was made up to five hundred cubic centimetres volume with pure

concentrated sulphuric acid, and used for addition to the blood samples submitted. This solution was found to be too weak giving no visible line of platinum at 2830 \AA . After running some experimental plates it was found that the most satisfactory solution was one containing .01 grams of platinum per 100 cubic centimetres of concentrated sulphuric acid. (Page 47. 7 and 8)

At this stage certain changes were made in the standard spectrographic conditions mainly to achieve easier measurement with reduction of the base spectrum background; these modifications were a change in the type of plate used, and an increase of the exposure time. The following are the standard conditions for blood analysis.

Instrument	- Hilger Spectrograph, large model.
Wave band	- $2,700 \text{ \AA}$ - $4,300 \text{ \AA}$
Slit width	- .015 millimetres.
Electrodes	- Upper:-(positive) H. and D. pure graphite rod 6.3 millimetres in diameter sharpened to a point at 80° angulation. Lower:-(negative) H. and D. pure graphite rod 10 millimetres in diameter cut in the shape of a crater to hold the ash.
Arc Gap	- 2 millimetres.
Current	- 110 volts D.C. 5 amperes.

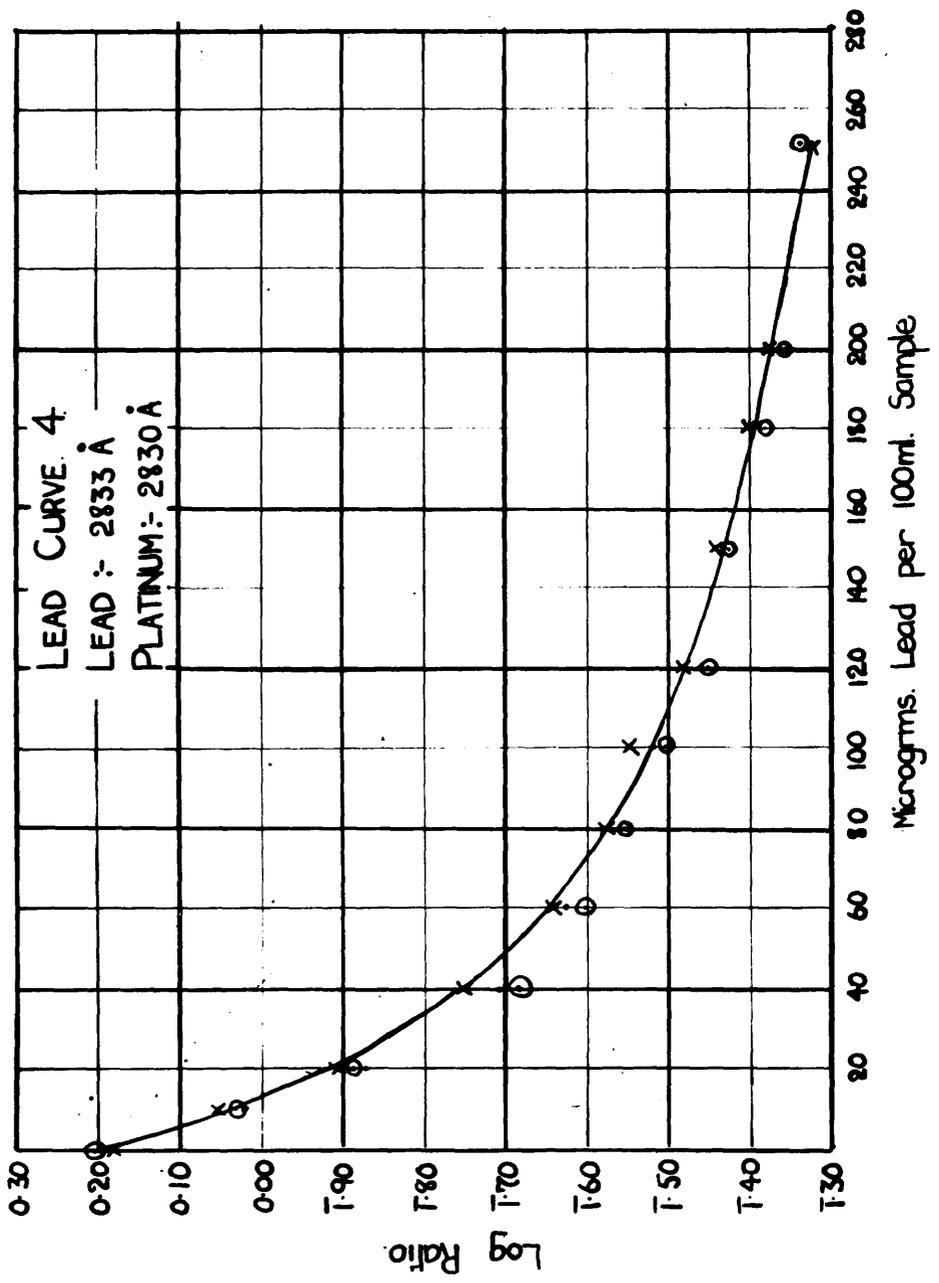


Plate - Ilford Ordinary, (H. and D. 70).

Exposure- One minute.

Development- a) Standard developer, 100 cubic centimetres
at 67°F. for 90 seconds.
b) Acetic Acid stop solution for 15 seconds.
c) Fix in Acid Hypo for 15 minutes.
d) Wash in running water for 15 minutes,
dry in a current of warm air.

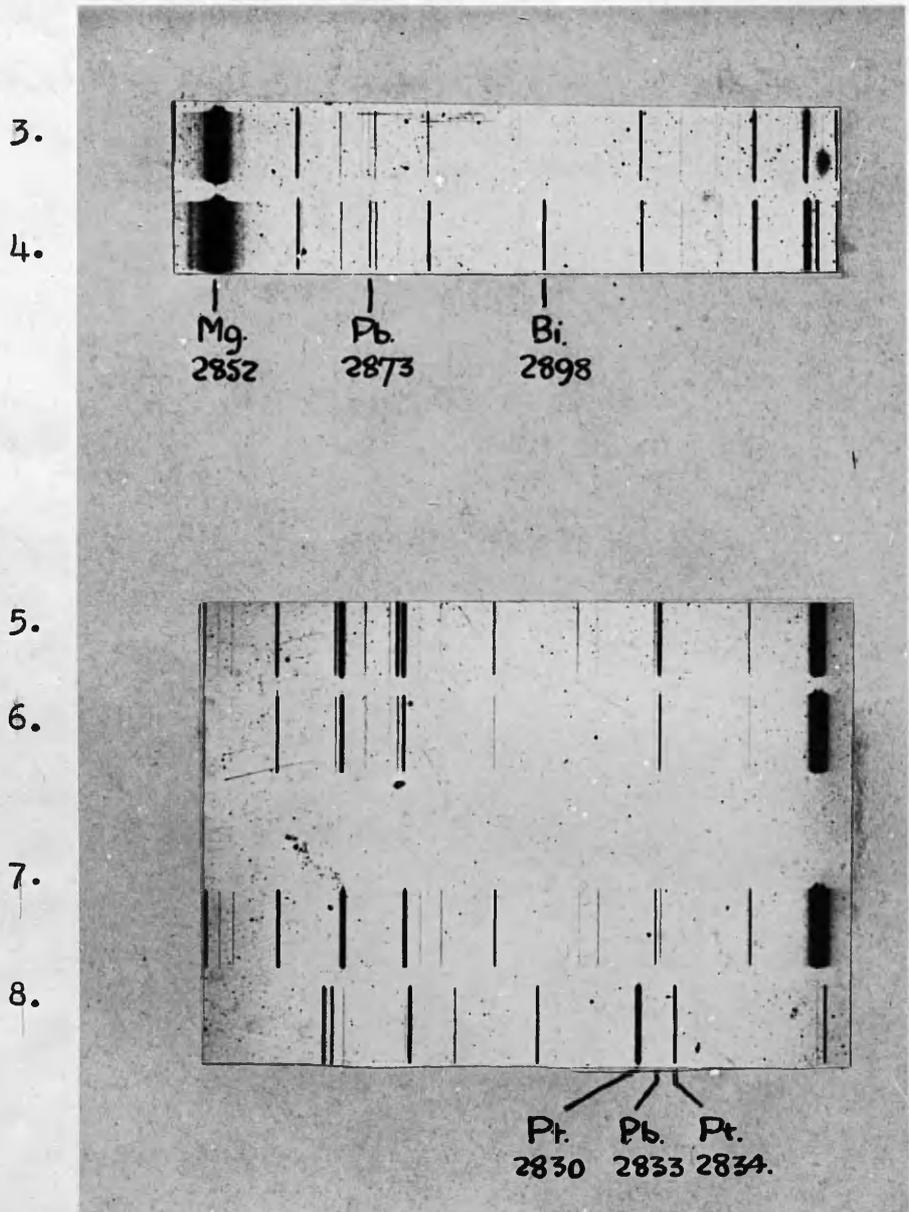
Using these conditions, a standard graph was obtained as for lead curves one, two and three, using platinum instead of bismuth as the internal reference element. The curve so obtained is shown in graph 4. Checking of the accuracy of this graph was achieved in the manner already outlined, and was found highly satisfactory. The use of this platinum line as an internal reference element is of some importance spectrographically. It fulfils all the conditions required of an internal reference element and can be used for other metals besides lead as is shown in Table III.

TABLE III.

<u>Metal.</u>		<u>Platinum.</u>	<u>Disease.</u>
Arsenic.	2860.5A ^o a*	2830A ^o a*	Acute and chronic Arsenic poisoning.
Nickel.	3002A ^o a*	2998A ^o a*	Nickel itch. Nickel carbonyl poisoning.
Chrome.	2986.4A ^o a* 3015A ^o a*	2998A ^o a*	Chrome holes. Chronic Nephritis.
Gold.	2676A ^o a*	2702.4A ^o a*	Therapeutic Control of Gold treatment.
Thorium.	2837A ^o a*	2830A a*	Aplastic Anaemia Pulmonary Neoplasm.
Vanadium.	3063.7A ^o b*	3064.7A ^o a*	Pulmonary tuberculosis, Haemolytic Anaemia. Haemoptysis.
Manganese.	2798.3A ^o a*	2830A ^o a*	Cerebro-spinal degeneration.
Cadmium.	2980.6 A ^o b*	2998A ^o a*	Acute Pulmonary oedema. Acute Nephritis. Acute Gastro-enteritis.

* Major lines notated "a".
Secondary lines notated "b".

In the consideration of Industrial Metallic Poisoning, these other metals are at present in obscurity; practically nothing is known of the range of their normal blood concentrations, or even if they are normally present at all.

BLOOD SPECTROGRAMS IN THE REGION 2.800/3.000 A°.

BLOOD SPECTROGRAMS IN THE REGION 2,800/3,000 A^o.

Spectrogram 3 shows a blood with bismuth absent.

Spectrogram 4 shows a blood with bismuth occurring normally.

Spectrograms 5 and 6 show normal blood with a marked difference in their lead content. The heavier concentration in spectrogram 5 is evident from the considerably more dense lead line than that of spectrogram 6.

Spectrogram 7 shows the synthetic blood ash with the lead line just visible.

Spectrogram 8 is a pure platinum spectrogram and shows that the platinum lines are in ideal position for measurement of the lead line 2833 A^o. Further, they are not overshadowed by iron to the presence of which the majority of the other lines in the spectrograms are due. The platinum line 2830 A^o is obviously more sensitive than that at 2834 A^o as can be seen from a comparison of their densities. Note how closely the spectrogram of synthetic ash corresponds to that of blood in all other respects except lead.

The accuracy of the method when different operators are spectrographing the samples under standard conditions resolves itself into their accuracy in:-

- I. Reading the deflection of the microphotometer scale.
- II. Calculation of the logarithmic ratios.
- III. Reading the graph.

The readings obtained by three different operators on a series of plates are shown below, and calculated in microgrammes of lead per 100 c.cs. sample.

TABLE IV.

<u>Spectrogram.</u>	<u>Operator.</u>	<u>Operator.</u>	<u>Operator.</u>	<u>Mean.</u>	<u>Error.</u>
No.	1	2	3	0	-
1	15	11	11	12	+ 3
2	13	14	13	13	+ 1
3	23	22	21	22	+ 1
4	24	25	23	24	+1
5	35	33	34	34	-
6	5	5	5	5	-
7	68	77	66	70	+7
8	40	40	40	40	-
9	5	5	5	5	-
10	5	5	5	5	-
11	5	5	5	5	-
12	5	5	5	5	-
13	210	200	210	207	-7
14	155	175	155	162	+13
15	62	62	59	61	-2
16	65	65	58	63	-2
17	16	17	15	16	-
18	200	200	200	200	-
19	7	7	7	7	-
20	11	11	12	11	+1
21	112	117	107	112	+5
22	70	80	72	74	+6
23	42	45	45	44	-2
24	28	27	26	27	+1

, It shows a very slight variation from individual to individual, (with the exception of three results), and is of no real significance.

In adapting the method to urinary analysis, it was decided to use the same method as for blood, i.e., by means of a synthetic ash. The ideal method for analysis of urine, would be to use as material for preparation of standards, urine which had been rendered lead-free. The task, however, of rendering large quantities of urine lead-free was given up, as it is laborious and a lead-free urine is never completely attained. Electrolytic deposition is the best method, but spectrograms of the urine after electrolysis showed persistent traces of lead.

(41)
Cooksey & Walton (1929), experienced similar difficulty.
(23)
(at the time the method of Bambach and Cholak (1941), had not reached this country). Spectrograms of urine ashes, however, revealed that the spectrum of urine and blood is identical. The difference is that there is a considerable reduction in the number of iron lines in urine; this reduction is a good example of what has been said before concerning 'a' and 'b' lines; the 'b' lines in blood are considerably reduced, or absent in a spectrogram of urine ash. This reduction of the number of lines gives what is technically known as a

weak base spectrum. Since the main base spectra of these fluids is identical it was considered feasible to use the lead curve 4 for the analysis of urine. Theoretically the weakness of the base spectrum of urine is a possible source of error, in that the weakness of its metallic content would lead to failure or weakness in its "arcability", with a consequent diminution of the reaction of the photographic emulsion on the plate.

Foster, Langstroth, and ⁽⁴²⁾McCrae, (1935), drew attention to the necessity of the components of a sample being characteristically the same, where it is desired to apply the same analytical procedure to samples of a different nature, and pointed out that a given weight of a metal when arced alone, would not necessarily give an emission spectrum of the same density, as the same amount when mixed in a sample containing other elements. This is understandable from the work of Breckpot, ⁽⁴³⁾(1937), who showed that in an arced sample of mixed metallic constituents, that the metals have a complementary effect on each other in the production of their characteristic spectrum lines. For practical purposes this objection does not hold in the present investigation as qualitatively blood and urine are identical as regards their metallic constituents -

the difference is quantitative; further by use of the method of internal reference, any alteration in arcing conditions due to polarity will affect both lead and platinum equally; this is proved by the results obtained from analysis of blank additions.

In its extreme form the weakness of the base spectrum of urine is manifested by failure of the arc once excited to "hold" for the standard exposure time, requiring repeated excitation. This difficulty while it can be overcome by extending the exposure time *pari passu* with the period of "dead arc" time resulting, led us to bring up the base spectrum of urine, by the addition of .02 grams of the synthetic blood ash which is lead free. This makes no difference to the result so obtained for the lead content of urine, and enables the standard conditions laid down to be more exactly maintained.

Similarly spectrograms of blood serum show a spectrum very similar to that of urine with a marked reduction of the iron lines of the blood samples; the other metals (copper, magnesium, zinc, etc.,) are, however, stronger than in urine, and generally the difficulty in arcing is not so readily encountered. It was accordingly decided to use the lead curve 4 for analysis of serum and urine.

The results of checks of the graphs on urine and serum are shown below.

TABLE V.

Case No.	Sex.	Sample.	Ash Wgt.	Microgms. added.	Result.	Recovery	Error.
34	Male	Blood	.0501	Nil	17	-	-
"	"	Serum	.0406	"	32	-	-
"	"	Urine	.0912	"	15	-	-
35	"	Blood	.0680	"	34	-	-
"	"	Serum	.0444	"	17	-	-
"	"	Urine	.1010	"	7	-	-
36	"	Blood	.0555	"	25	-	-
"	"	Serum	.0422	"	32	-	-
"	"	Urine	.0988	"	15	-	-
34	"	Serum	.0410	20	47	15	-5
35	"	Serum	.0456	40	60	43	+3
36	"	Serum	.0430	60	97	65	+5
37	"	Urine	.094	Nil	5	-	-
"	"	Urine	.094	10	20	10	+5
39	"	Urine	.096	Nil	3	-	-
"	"	Urine	.096	20	27	7	+4
41	"	Urine	.092	Nil	7	-	-
"	"	Urine	.092	20	23	3	-4

A further modification in the analysis of urine I consider desirable. In the process of ashing samples of urine, on elevation of the temperature the sample tends to spurt, and boil over leading to loss of material. This is largely overcome by the addition of one gram of Dextrosol D. Glucose, (lead-free) which tends to act as a binding agent. Urine further tends on ashing to fuse to the side of the crucible resembling a hard crystalline salt, the addition of the glucose prevents this and renders the ash easier to handle. Do the additions outlined make much difference to the accuracy of results? Checking of samples with and without additions has shown that the error is usually not more than ± 3 microgrammes per 100 cubic centimetres and never more than 8 microgrammes per 100 cubic centimetres of urine.

TABLE VI.

Sample.	Addition.	Result in microgms. Pb/100 c.cs. Urine.	Variation.	Mean.
A	5c.c.Urine Nil	40		
A2	" " .02 gms. Synthetic ash.	48	8	44
B	" " Nil	20		
B2	" " .02 gms. Synthetic ash.	20	Nil	20
C	" " Nil	5		
C2	" " .02 gms. Synthetic ash.	12	7	8
D	" " Nil	18		
D2	" " .02 gms. Synthetic ash.	13	5	15
E	" " Nil	10		
E2	" " .02 gms. Synthetic ash.	18	8	14
F	" " Nil	18		
F2	" " .02 gms. Synthetic ash.	14	4	16

This probably results from slight over and under exposure due to miscalculation of "the dead arc period". The error is insignificant even when it is considered that the normal urine lead is low. The addition of the Dextrosol D. could be dispensed with under exact and careful supervision of the reducing and charring process; it is my own practice to dispense with additions as not making such a difference to the result obtained as to be serious. McPheat prefers the additions to be made, and it must be admitted that his results, as checked from blanks, are of the highest degree of accuracy. On the other hand for the slight gain in the accuracy of the result, a critical examination of the urine from the relationship of one metal to another is forfeited by the addition of these metals in the synthetic ash; one might be desirous of appraising and measuring, as to whether any sharp increase in the urine lead caused an increased or diminished excretion of another metal, e.g., copper, it cannot be done if the additions are made, further it violates one of the cardinal axioms of micro-analysis, namely, that the fewer reagents used, and the less the sample is handled, the less the risk of contamination error; thus it is possible that by flooding the spectrum

to abolish a minus error, an error of an amount equal to the quantity it is desired to abolish is propagated the other way. It is always possible in the case of urine to estimate two samples, with additions and without, and take the mean of the results so obtained, the result will probably be 100% accurate. It was not considered necessary or advisable to make additions for estimations on serum, as iron is the only element to show marked reduction.

Cerebro-spinal fluid does not show much variation from serum or urine when subjected to spectrographic examination. A sharp reduction of the iron lines is the most striking feature. In spite of the fact that it is the most difficult ash to handle spectrographically, no additions were made in the expectation that this fluid would show the greatest trace element variation, in accordance with the pathological condition for which lumbar puncture was made; although the lead content was the only one under examination, it was decided not to forfeit the opportunity of observation on the other trace elements. From its low ash weight, as one would anticipate, it is difficult to handle spectrographically, and requires careful watching for "dead arc time" periods.

I have not as yet obtained sufficient quantities of cerebro-spinal fluid on which to check the accuracy of lead curve 4. At present I must assume that since the main base spectrum is identical to that of serum and urine, the error will not be of appreciably greater magnitude.

It was anticipated that there might be some difficulty in the application of the method to the analysis of the tissues content, namely, from the difficulty of reduction to a suitable ash. This difficulty I found non-existent, as blood is the most difficult of all biological material to ash yet encountered. The main base spectrum of all the tissues corresponds with blood. In the case of Hair and the Nails, the method is also applicable, but so far I have only considered its qualitative application to these tissues.

SECTION V.SOURCES OF ERROR.

In considering the possible sources of error in the method, it must constantly be borne in mind that the quantities dealt with are extremely small, and that next to iron, lead is probably the most ubiquitous of all the elements. The danger of contamination error is considerable, and even in its minimal form may seriously affect the result. Contamination errors may arise in:-

- A. The preparation of the material for spectrographing.
- B. In spectrographing the sample itself - the least serious of the two sources of error.

The sources of contamination error in group "A" may be listed as follows:-

- (I) From the antiseptics used in cleansing the patient's arm.
- (II) From the needle and the syringe.
- (III) From the crucibles and lids used for collection of the blood.
- (IV) From the Sulphuric Acid used for sulphation.
- (V) From the furnace used for ashing the blood.
- (VI) From the balance on which the ashes are weighed.

The disposal of these difficulties is achieved as follows, although it will be seen that some of them are insurmountable.

- (I) The patient's arm is cleansed with lead-free absolute alcohol, and no other antiseptic is used.
- (II) The Pyrex syringe and needle, are sterilized in a sterilizer from which the water has been shown by spectrograph to be practically lead-free.
- The needle once the blood has been aspirated is immediately screwed off the syringe, and the blood transferred at once to a lead-free silicon vitreosil crucible, and covered with a lid of the same material at once. So far we have been unable to obtain a guaranteed lead-free needle, and this is one source of contamination error. Blood, however, is not of such an acidity that in the short period of contact, there should be any chemical reaction with the metallic constituents of the needle.
- (III) The crucibles and lids are guaranteed lead-free, and boiled in concentrated Hydrochloric Acid, and washed in distilled water immediately prior to use.
- (IV) The blood is then sulphated in the crucible without transference; while the sulphuric acid used is lead-free, the glass pipette is not, and provides us with a second insurmountable possible source of

contamination.*

(V) The sulphated samples are now transferred to the furnace, which is dust-proof, and completely closed, being only opened to load or withdraw the samples. It is possible that minute contamination may occur here, from the fine brick dust arising after prolonged use, by reason of the expansion and contraction of the furnace, and the presence of acid fumes, but it cannot be estimated, and if there be contamination all samples should be equally affected; it is useless to cover the samples during ashing, as the acid fumes must be allowed to escape. It is better to insert the crucibles into a cold furnace by hand, rather than by tongs into a furnace at the first reducing temperature; this eliminates the risk of the acid contents of the crucible contacting the metal edge of the tongs; after the ash has been attained, the volume is so reduced that there is no danger of the tongs contaminating the ash.

(VI) The ashes obtained are weighed in a Periodic Prismatic Reflecting balance, the holder of which is made of Pyrex, and with care no contamination risk arises here; the ashes can be transferred to

* We have since obtained a Pyrex pipette.

the holder by gentle tapping, and vice versa without contact with any other instrument.

For serum there is as yet no Pyrex or Silicon centrifuge tubes, and common centrifuge tubes must suffice. The sample is covered with a number 41 Whatman filter paper prior to spinning, and the serum aspirated into the Pyrex blood syringe, measured to five cubic centimetres, and treated as above. Urine may be collected in a Pyrex flask, or beaker, aspirated into the syringe, measured, and transferred to the crucible as described. Contamination in both males and females may be present in the form of dust, talcum powder etc; the patient should, therefore, be instructed to pass urine, and the last of the sample collected for analysis. A useful method particularly in males, is to provide them with an ordinary basin, and a clean crucible, instructing them to pass the first urine into the basin, and direct the rest into the crucible, the overflow passing to the basin; five cubic centimetres are aspirated from the crucible, and transferred for analysis to a second clean crucible, The possible sources of error are few, and probably of very slight significance; it will be appreciated that the sample before and after collection, is subjected to the

minimum of reagents and handling.

GROUP "B".

The possibility of contamination error during spectrographing lies in the use of contaminated electrodes; this is ruled out by grinding the arc ends of the electrodes before use, any contamination present being dissipated with the graphite dust; spectrographing of the electrodes before use has always shown them to be lead-free.

INEXACT SAMPLE VOLUMES.

An inexact quantity of sample especially in comparison work is a source of error also, and is most marked when different individuals draw the samples, e.g., in submission of a sample from hospital for investigation. This is due to faulty calibration in syringes, no two being exactly alike. Air bubbles and froth, also tend to an amount less than five cubic centimetres being drawn. The first source of error can be considerably checked by always weighing the samples obtained before ashing, or the ashes before spectrographing. Blood ash from samples of five cubic centimetres usually varies between .05 to .06 grams, and ashes may be obtained weighing slightly above or below this range. The same applies to Serum (.025 to .04 grams.); wide variation from the common range of sample or ash weights found, should

always be suspect, and if possible checked by a further sample.* In the Weir laboratories the same syringe is always used, and the variation of ash weights is very low; I have occasionally encountered wide variations in samples received from outside, but it cannot be overcome, and it may be argued that for comparison work where the same instrument is used for different body fluids, the error applies equally to all. Aspiration of more than the required five cubic centimetres of blood allows of expulsion of air, and exact quantities being measured, especially where the needle is removed prior to expulsion of air.

The ashes can be transferred to the crater of the cathode, by gentle tapping on the base of the crucible; any further source of error is spectrographic in origin and is completely eliminated by strict adherence to the standard conditions laid down; these possible errors will be discussed as they arise in the following paragraphs.

One point of importance should be stressed, all apparatus from syringes to electrodes, should be used exclusively for the particular investigation under consideration.

* Silicon vitreosil crucibles are not made of standard weight and require weighing and numbering before use.

if contamination errors are to be reduced to a minimum.

SOME SPECTROGRAPHIC CONSIDERATIONS.

SECTION VI.

CHOICE OF WAVE-BAND:-

Stress has been laid on the necessity of strict adherence to standard conditions, as they vitally affect the accuracy of working. Reference has been made to the mechanism by which the prism and lenses are adjusted, in accordance with the field of the spectrum in which it is desired to work. The range of the spectrum covered by the large Hilger spectrograph is from 1910 \AA° to 8000 \AA° , and for our purposes may be arbitrarily divided into three zones. The lower zone from 1910 \AA° to 2700 \AA° , a mid zone 2700 \AA° to 4300 \AA° , and an upper zone 4300 \AA° to 8000 \AA° . Examination of the lower zone shows that the lines are few in number and widely dispersed, in the high zone the lines are profuse and too closely packed; these facts are such that they render internal reference measurement difficult; further, in the high zone (6000 \AA° upward), the field is occupied by the red spectrum, and working at this wavelength has special difficulties entailing the use of Panchromatic plates, and development in darkness. In the mid zone 2700 \AA° to 4300 \AA° , (the ultra violet, violet region), the spectrum lines are numerous, and dispersed there satisfactorily from the point of view of measurement, (neither too close nor too

far apart); in other words there is present the maximum dispersion for the maximum number of lines of most of the elements, which are reasonably discrete, and not overshadowed. Where possible, I always endeavour to work in the mid zone. Certain elements are only detectable in the lower or upper zones e.g., mercury (2537 A°), gold (2676 A°), uranium (4242 A°), and even where the internal reference element is the same, standard graphs must be plotted in accordance with the governing standard conditions, one of which is the wavelength within which it is desired to work.

OVERSHADOWING AND CHOICE OF ELECTRODES.

The biological application of spectrography presents difficulties which are not encountered industrially. One of the worst is the spectrographic sensitivity of iron, an element constantly present in biological material. Elements vary in their spectrographic sensitivity, so that weight for weight some elements might emit several hundred lines (iron), others only two or three, (uranium, gold), when spectrographed under the same conditions. Iron is highly sensitive spectrographically, and emits several hundred lines in low concentration. The result on the developed plate of a biological sample, is, that the lines of the other elements tend to be "overshadowed" by the ubiquitous iron lines. Another example of overshadowing

is found in the mutual overshadowing of sodium and zinc at 3302 \AA . This overshadowing in no way affects the principle previously stated, namely, that the waves of light emitted by an element in a state of atomic excitation are of a wavelength specific to itself, and no two elements will occupy the exact same site on the spectrograph field; in the exhibition of overshadowing, the juxtaposition of the lines is so close that the eye cannot separate them, although a difference in the spectrum is evident when one or other element is absent. This phenomenon of overshadowing may, therefore, render impractical of use many a line which appears useful on consultation of spectrograph charts, or wavelength tables. Other elements with this tendency to overshadow, are magnesium and copper, although not so prominent as iron.

Another source of overshadowing may be encountered from the electrodes; for this reason, with few exceptions for biochemical investigations, the best electrodes are made of spectrographically pure graphite. They are obtainable in a state of high chemical purity, and are readily cleaned after use. These advantages far outweigh the drawback of the dense cyanogen band they emit between $3684 \text{ \AA} - 3889 \text{ \AA}$ and $4060 \text{ \AA} - 4124 \text{ \AA}$, due to the formation of cyanogen from the interaction of the

atmospheric nitrogen, and the carbon of the rods to form CN. If it were desired to examine any element in the cyanogen region, it could be achieved by the use of silver, or copper electrodes; these latter have the disadvantage that they are much more costly, and are difficult to clean. The reason is not clear, but the spectrographed elements tend to penetrate deeply into the metallic electrodes, so that filing and scraping for about ten minutes is necessary before they are free of contamination; by reason of it's softness, a graphite electrode can be cleaned in about fifteen seconds with an instrument similar to a pencil sharpener, and does not allow of permeation. When a large number of samples has to be done, the saving of time and labour can be readily appreciated.

"a" AND "b" LINES.

Contrasting a developed plate of blood, and urine, an outstanding difference at once strikes the eye; although spectrographed, and developed under identical conditions, a large number of lines in urine have been sharply reduced in density, a few have disappeared; this is most evident in the case of the iron lines. The explanation of this phenomenon is to be found in the work of De Gramont already referred to. The amount of iron in urine is so reduced that it is only "Les raies ultimes" which persist, and

indicate the reduction of the concentration of the element of origin in urine. It is seen, therefore, that just as some elements are spectrographically more sensitive than others, each element has lines which are more sensitive for its qualitative and quantitative detection. In deciding on the choice of a line on which to work, this factor has to be borne in mind; some elements are toxic in extremely low concentrations, and if one is tempted to use a "b" line by reason of lack of overshadowing, or proximity to the internal reference element, one may well find that no line is visible on the developed plate at the wavelength site chosen; all that could be concluded from such a result, would be that the element was not present in the sample above a certain concentration, dependent on the concentration at which the "b" line is no longer reproduced. The use of a "b" line, therefore, is not practical except where the element is of sufficient spectrographic sensitivity, to emit "b" lines below the concentration it is anticipated to meet. Lead is of sufficient spectrographic sensitivity to reproduce an "a" line in concentrations corresponding to .000001 grams. per 100 cubic centimetres of blood. It is, therefore, convenient to use for quantitative analysis

This consideration does not apply to the internal reference element which is always being added in a fixed amount to the sample, its reproducibility having been previously ascertained for such a concentration.

THE INTERNAL REFERENCE ELEMENT.

Other considerations, however, govern the use of the internal reference element; these may be stated as follows:-

- I. It should be an element not normally present in blood or tissues, and an unlikely constituent even under extremely improbable conditions.
- II. The internal reference line it is desired to use should be reasonably close to the chosen line of the element under examination.
- III. It should be discrete, and not overshadowed.
- IV. It should be capable of introduction to the sample in the sulphuric acid used for its sulphation.
- V. The solution of the internal reference element in sulphuric acid should be stable and not precipitate with keeping.
- VI. The constant amount added should reproduce sufficiently on the photographic plate to give such a deflection on the galvanometer scale as to be measurable, yet it should not be so dense that its deflections are beyond the measurable range of the galvanometer scale.

I consider that the normal presence in blood of the internal reference element is the most likely source of error; admittedly bismuth which I discarded for this reason is present in blood in very minute quantities, and the presence of microgramme amounts per hundred cubic centimetres would be infinitesimal in terms of five cubic centimetre samples, and, at first sight, should not seriously affect the result. It should be noted, however, that the amount of bismuth used as an internal reference element is very small (5.6 microgrammes), when compared with the amount used for platinum, (.01 grams.) so that microgrammes of bismuth already present in the sample would tend more seriously to throw out the result; in other words, bismuth is of such spectrographic sensitivity that only very small additions are necessary to increase the density of its emission spectrum. Further, to judge by the literature, bismuth itself is frequently contaminated with lead.

In perusal of the literature for choice of an alternative element, I found that I was not original in the use of bismuth as an internal reference element for lead. ⁽⁴⁴⁾Cholak, while varying his preparation for ashing, used bismuth for this purpose. In his recent extensive comparison of analytical methods of lead

analysis (Feb. 1943), it must be admitted that from his results, the danger of a bismuth base error does not seem to be great; nevertheless it is there, as I have proved, and when bizarre results are encountered tends to cause uneasiness, as to the source of the departure from the usual range of result; for this reason I adopted platinum as the internal reference element which incidentally has the advantage of a much wider range of application.* Daniel, Houston, and Kees⁽⁴⁵⁾ (November 1942) have described a method for detection of Vanadium (3185.4 A°), by use of a synthetic ash, and chromium (3188 A°), as the internal reference element. The work was done using rats as experimental animals but much of its value is lost when the results are applied clinically. Chromium is not infrequently encountered in human blood from occupational sources, and vanadium is frequently associated with it. Kent and McCance⁽⁴⁶⁾ (1941), using a medium quartz spectrograph for the estimation of lithium, boron, gold, silver and vanadium, used manganese and iron as the internal reference elements; this entailed estimating the amounts of these elements already present in the sample, a procedure which by increased use of chemical reagents and operations, markedly

* Cholak's results, (1943) show mean blood lead concentrations which, in our opinion, are too low, (30 microgrammes per hundred cubic centimetres).

increases the risk of error; further the analysis is rendered slow and laborious, losing a considerable advantage of the spectrographic method, i.e., speed of working.

At first sight the amount of sulphuric acid used may seem excessive for the sulphation of five cubic centimetres of blood; Blumberg (1934)⁽⁴⁷⁾, used 1.5 c.c.s. of sulphuric acid for purposes of sulphation; my experience with this quantity of sulphuric acid was that sulphation was incomplete; the ashes were white in colour with no evidence of the rusty tinge denoting iron, examination of the developed plate showed an almost complete absence of the prominent Magnesium lines - a condition incompatible with life.

The considerations with regard to overshadowing of the internal reference element are obvious; if overshadowing is present it is impossible then to say to what degree the deflection on the microphotometer scale is due to the overshadowing element. Nevertheless, the possibility is often overlooked, and, as I pointed out (1943)⁽⁴⁸⁾, seriously affects the validity of the results on gold excretion reported by Kent and McCance (1941).

PLATE ECCENTRICITY.

Under war time conditions, especially, a plate is met with occasionally on which the photographic emulsion is

not evenly distributed; the result is that different parts of the plate exhibit variations in their response to light to produce darkening of the plate; this is known as "Plate Eccentricity". It is for this reason that it is desirable that the internal reference line, and that of the test element should be in as close proximity to one another as possible; if Plate Eccentricity be present both elements are then affected equally and no difference to the result occurs.

EDGE LINES.

It is quite practical where standard graphs have been plotted for each element, to measure the quantity of a number of elements on the one plate; this would be quite possible in the region $2700 \text{ \AA}^{\circ} - 4300 \text{ \AA}^{\circ}$, using platinum as the internal reference element (Table III); some consideration should be given to the "Edge Lines" as their use will be considered. When plates are made, they are cut from long strips to the required length (21.6 centimetres); the emulsion at the edge of the plate is, therefore, subject to tension in the cutting process, and may exhibit features of eccentricity. In its extreme form this is encountered in the form of Emulsion Run, the emulsion running on development. Gold, for example, emits an "a" line at 2676 \AA° . and platinum an "a" line at 2830 \AA° ., so that by plotting a standard

graph for gold against platinum, gold, if present, could be measured on the same plate as lead.

$$\left(\begin{array}{cc} \text{Au. } 2676 \text{ A}^\circ & \text{Pb. } 2833 \text{ A}^\circ \\ \text{Log r. } \frac{\quad}{\text{Pt. } 2830 \text{ A}^\circ} & \frac{\quad}{\text{Pt. } 2830 \text{ A}^\circ} \end{array} \right)$$

Unfortunately the gold line is right at the edge of the plate, and is classed as an edge line. In the absence of "emulsion run", it is difficult to say what is the effect of "edge tension" on results, and requires further investigation. At present it is better to raise or lower the wavelength field, and plot graphs in accordance with standard conditions; accuracy of results will always then be certain. From the multiplicity of precautions listed, it might seem that the method contains many factors liable to erroneous results, it is not so, however. The majority of them are encountered very occasionally, e.g., in several hundred plates I have only once seen "emulsion run"; I have described them as the possibilities, rather than probabilities which one may encounter with wide working experience. It is evident that adherence to standard conditions is absolutely essential for accurate working.

DISCUSSION.

At the risk of tedium, the necessity for standard conditions has been stressed. This is imperative because

of the sensitivity of the instrument, and the delicacy of the method. The use of the instrument, and its accessories, may be criticised on the grounds of its cost, and that its use is limited. No doubt the same arguments were advanced when the first X-Ray outfit was designed, and Electro-cardiography first introduced; that has not prevented these instruments becoming essential adjuvants to diagnosis. Spectrography has only come into its own in the past ten years, and its application becoming increasingly popular. It is such that few industrial organizations of repute do not employ it in their research armamentarium. Is the field of application of the instrument too limited? In this connection it is now generally accepted that the trace elements have a physiological function of which we are at present in ignorance; an ignorance begotten of the lack of an analytical method of sufficient sensitiveness, and accuracy. There are wide research fields for the use of the Spectrograph in assessing the variations of the trace elements in health and disease; the Anaemias for example, present one pathological group in which much could be learned from spectrographic analysis. Of particular interest, and the main purpose in the elaboration of the method, is its application to Industrial Metallic Poisoning. Lead is practically the only heavy metal of which we know

something of the toxic concentration. Manganese is credited with toxic symptoms of an extremely grave character, yet the limit of its concentration in the circulation, or indeed if it be normally present at all, is unknown. The present day attempts at control of the grave industrial exposure to radio-active substances is checked by blood counts. Considering that the effects of absorption do not manifest themselves for several years afterwards, the attempts at control do not seem adequate. Spectrographing of blood samples from such exposed workers, would readily reveal the absorption into the circulation of the toxic agent, (Mesothorium), and as a routine check, would be of considerably more value than blood counting.

The absorption and excretion of drugs could be studied by spectrographic analysis of the blood, more exactly than by an empirical dosage based on age and body weight, especially where overdosage may produce toxic symptoms as is the case in gold or arsenic therapy. All these problems remain to be investigated; that they have not already been done, has been due to the lack of a suitable analytical method; there is no doubt that the method described for lead analysis, and the work of other workers

points the road along which such investigations lie.

The spectrographic method described for lead analysis has much to commend it over the chemical methods hitherto in use; and further shows advances over the spectrographic methods elaborated by other workers.

OPERATIONAL TIME.

The operational time required for the spectrographic method presents distinct advantages over chemical procedures. The main time lag is during the process of reduction and charring. The actual exposure of the plate, developing, viewing, and measuring, occupies about one hour for one sample. It is seldom, however, that one confines oneself to a single sample; I have exposed, developed, viewed and measured twelve samples on the same plate in two and a half hours. The presence of numerous different samples on the one plate, apart from the convenience of having them all developed at the same time, is advantageous in that it affords the opportunity of comparison of the individual's different body fluids on the same plate. The samples once sulphated, can be placed in the furnace and left to themselves, leaving the operator free to do other work; the necessity of constant supervision is thus eliminated. Comparing this operational time with that of Kehoe's method, (modified

Fairhall technique), where the single estimation is protracted over three days, or even the faster method of Tompsett (twenty-four hours for a single estimation), the advantages of reduced operational working are obvious.

CONTAMINATION ERROR.

The greater the number of chemical reagents used, the greater the risk of contamination error, and such contamination errors vitally affect the accuracy of results (vide McKee's observations on the work of Abraham and Baird⁽⁴⁹⁾). Comparing the sources of chemical contamination in the spectrographic method, with the multiplicity of reagents used in the chemical procedures, it will be seen that with the former, contamination from this source is practically non-existent.

The results obtained may be further affected by frequent handling of the samples, and loss in transference from one vehicle to another, particularly where a large number of chemical manipulations are involved. In the spectrographic method this source of erroneous result is entirely eliminated.

AMOUNT OF ANALYTICAL MATERIAL.

Few workers there are, who have not experienced difficulty in obtaining the analytical material for

examination, even with the full co-operation of the patient; patients are less co-operative when large amounts of blood have to be withdrawn, and usually object to repeated examinations. The quantity of material necessary for spectrographic analysis, permitting as it does repeated examination even in the course of a day, is of advantage both to the patient and operator. Five cubic centimetres of blood is all that is necessary, and different body fluids may be estimated in the same amount. By comparison, Kehoe required fifty cubic centimetres of blood, and a litre of urine for his investigations. Tompsett's method requires much less, (twenty cubic centimetres of blood), although still on the high side where repeated examination is necessary. Obviously Kehoe's method is inapplicable to the analysis of serum as it would require about 150 cubic centimetres of blood to give the required amount of serum; Tompsett's method, while of practical application to serum analysis, requires rather a large amount of blood to give twenty cubic centimetres of serum and repeated examination is not practical. I cannot estimate the amounts required for analysis of cerebro-spinal fluid by these methods, but I cannot trace any such analysis in Kehoe's exhaustive studies, nor as yet have I found a report

by Tompsett on the lead content of cerebro-spinal fluid. The advantages of the use of such small quantities of blood are many times magnified when the subject is a young child, whether it be from the point of view of collection of blood, or collection of urine.

SENSITIVITY OF METHOD.

In spite of the use of such large quantities of blood, Kehoe occasionally failed to detect lead; this, in my opinion, denotes a lack of sensitivity in his methods. On only one occasion have I failed to find a lead line at 2833 A° in a blood analysis; it is of interest to record, however, that the blank space on the plate at 2833 A° when moved across the microphotometer slit did show a small deflection; subsequent examination of the plate microscopically revealed that a faintly perceptible line was present. The method is highly sensitive qualitatively, and accurate where standard conditions are observed to .000008 grams per hundred cubic centimetres of blood and urine.

PERMANENT RECORDS AND INDIVIDUAL ERROR.

Two additional advantages exclusive to the spectrographic method are firstly, once spectrographed, a permanent record of the result is provided for

comparison with future work; secondly the possibility, (or probability), of human error is reduced to a minimum, once set, the machine does the work. In the chemical methods, apart from risks of analytical error, the result depends on a colourimetric comparison with standards; this is liable to be affected by extraneous factors such as lighting, to say nothing of the fact that no two individuals' colour perception is alike.

ADVANCE OVER PREVIOUS SPECTROGRAPHIC METHODS.

Of the few who have adopted the spectrographic method of analysis, Cholak and Blumberg have been foremost, Cholak's procedure, using the internal reference method and microphotometer measurement, I prefer to Blumberg's estimation against standard solutions by a visual comparison of line densities. I have already criticised Cholak's use of bismuth as the internal reference element. I believe that the use of platinum as the internal reference element will prove of considerable value for the spectrographic analysis of other metals, apart from the fact that it is the internal reference element of election for lead analysis.

The elaboration of standard graphs from a synthetic

blood ash now proven accurate, will confer a great advantage for future work. The preparation of graphs for gold, silver, nickel, arsenic and thorium will be possible by adding increasing quantities of the element under investigation to .05 grams of the synthetic blood ash, and adding the fixed amount of platinum in sulphuric acid. It is wise, therefore, to prepare a quantity of synthetic ash far in excess of normal requirements as a reserve for future work. It is a relief to know that I am not likely again to encounter the difficulty of obtaining pure reagents; I cannot visualize impurity in supposedly pure substances from gold, nickel or arsenic. Much work yet remains to be done on the application of the spectrograph to the analysis of biological material.

I am optimistic that the amount of blood required for analysis will ultimately be reduced as to render venipuncture unnecessary, as the potentialities of sensitive plates and increased exposure times become more thoroughly understood.

PART 2.

By reason of the numerous analytical procedures used in the determination of lead concentrations in biological materials, the diverse results reported from the hands of competent observers have rendered the subject one of considerable controversy. From a study of the subject, I concluded that the investigations outlined were necessary in an attempt to obtain more exact knowledge of the biochemical behaviour of lead in the body.

SECTION I.THE HEREDITARY FACTOR.

It was soon evident that the range of normal blood lead concentrations was fairly wide, complicated further by considerable variations in groups of individuals with equal industrial exposure to lead absorption. This was clearly brought out by examination of a sample of blood, from a case of lead poisoning submitted for analysis from a large city hospital. The result of the analysis showed the patient to have a blood lead lower than several of our own normal cases, (50 microgrammes per 100 cubic centimetres);

it had to be admitted that in this case the clinical criteria for a diagnosis of lead poisoning were very slender, but the result was repeated later in a case with much more clearly defined clinical findings, e.g., lead line, abdominal pain, and paresis of the wrist; the result in this case confirmed by dithizone estimation at the hospital was seventy microgrammes per hundred cubic centimetres of blood.

(50)

Legge and Goadby, (1912), were of the opinion that a hereditary susceptibility to lead poisoning exists. They quote the case of a father and three daughters, who all developed lead poisoning in employment where no other workers were affected; also the case of two brothers who developed lead poisoning although exposed to exactly the same risk as their fellow workers who exhibited no symptoms; a third brother subsequently employed, and exposed to a minimum of lead risk by reason of the known family history, rapidly developed the symptoms of lead poisoning, although more adequately protected than his fellow workers.

It is a fact of old standing recognition that still births are not infrequent where the mother has been unduly exposed to lead during pregnancy. Oliver, painting eggs with lead nitrate found thereafter the embryo chicks in a state of arrested development.

It was accordingly decided to investigate the blood lead content of newly born infants, for comparison with that of the maternal circulation in particular, and adult concentrations generally. The samples for analysis were submitted by Dr. D. McKay Hart, of the Maternity Hospital, Glasgow, and collected as follows:-

Five cubic centimetres of blood were withdrawn from the maternal circulation in the second stage of labour, just prior to delivery of the foetus; five cubic centimetres of blood were withdrawn from the umbilical vein prior to severing the cord, as representative of the foetal circulation. The samples were immediately transferred to silicon vitreosil crucibles, covered, and spectrographed under standard conditions. The results are shown below.

TABLE I.

Mother	A.	54	microgrammes	per	100	c.cs.	of	blood.
Baby	A.	51	"	"	"	"	"	"
Mother	B.	43	"	"	"	"	"	"
Baby	B.	42	"	"	"	"	"	"
Mother	C.	34	"	"	"	"	"	"
Baby	C.	28	"	"	"	"	"	"

From these results it was clear that the blood lead concentration of newly born infants approximates closely to that of the mother, and is in some cases identical. The high lead content of the foetal circulation is surprising, approximating as it does so closely to that of the mother, and indicating that little or no lead is prevented by the placenta from transmission to the foetus; as far as can be judged from a visual comparison of line densities, this is not exclusive to lead, all the metals in the maternal circulation being transmitted in much the same concentration to the foetus. On reflection, this is not so surprising as may appear at first sight; the hormone molecules, much larger and more complex than those of the metals, pass the placenta freely, and the efficacy of the antenatal treatment of syphilis indicates free placental transmission of arsenic. (51)

This is confirmatory of the observations of Porak, (1889), and Calvery, (1938), (52) although exception may be taken to Porak's description of the trace elements as "des substances étrangères à l'organisme", implying that they are not normally present. From these comparison results, it is clear that the maternal

circulation plays a significant part in determining the blood lead level of the child, and raises the question as to why a metal, hitherto regarded as purely foreign to the blood stream, and capable in excess concentration of producing grave toxic effects, should be transmitted in such large amounts relative to the foetus; if anything since the blood volume of the infant is higher than that of the adult, the whole blood lead concentration of the infant is greater than the mother's. It was hoped to obtain samples of blood from the same infants at the end of the first week of life, to ascertain if the lead so inherited was retained or excreted; this unfortunately was not possible owing to difficulty of collection. The excretion of lead in the infant urine, however, gives some indication as to whether the lead acquired from the mother is retained; here again difficulty of collection was encountered, and the labour involved negatives examination of a large series. The samples of milk were also analysed in an endeavour to ascertain the amount of lead available for absorption by the infant.

The results are shown below:-

TABLE II.

Results in Microgms/100 mls.

	<u>Mother Blood.</u>	<u>Mother Milk.</u>	<u>Baby Blood.</u>	<u>Baby Urine.</u>
D.	46	32	71*	2
E.	22	8	37	7
F.	33	20	18	3
G.	33	5	25	-

The samples of milk and baby urine were drawn on the fifth day. Lochial contamination negatives examination of the maternal urine, except by catheter specimen.

The samples of blood were collected as in Table I. The infant urines were collected in special silicon vitriosil test tubes, strapped in position for collection of urine, the samples of milk were collected where possible by direct expression into the crucibles; it was not always possible to obtain an exact quantity of either samples, but enough was obtained to give a fairly accurate analysis. From these results it will be seen that there is no evidence of a high lead excretion in the infant, and the conclusion must be reached that the lead inherited from the mother is largely retained. There is a sufficient amount of lead in human milk to account for the amounts which appear in the infant's urine, and, as will be seen later, the ratio of blood content to urine content in the infant, is similar to

* Doubtful result, probable contamination.

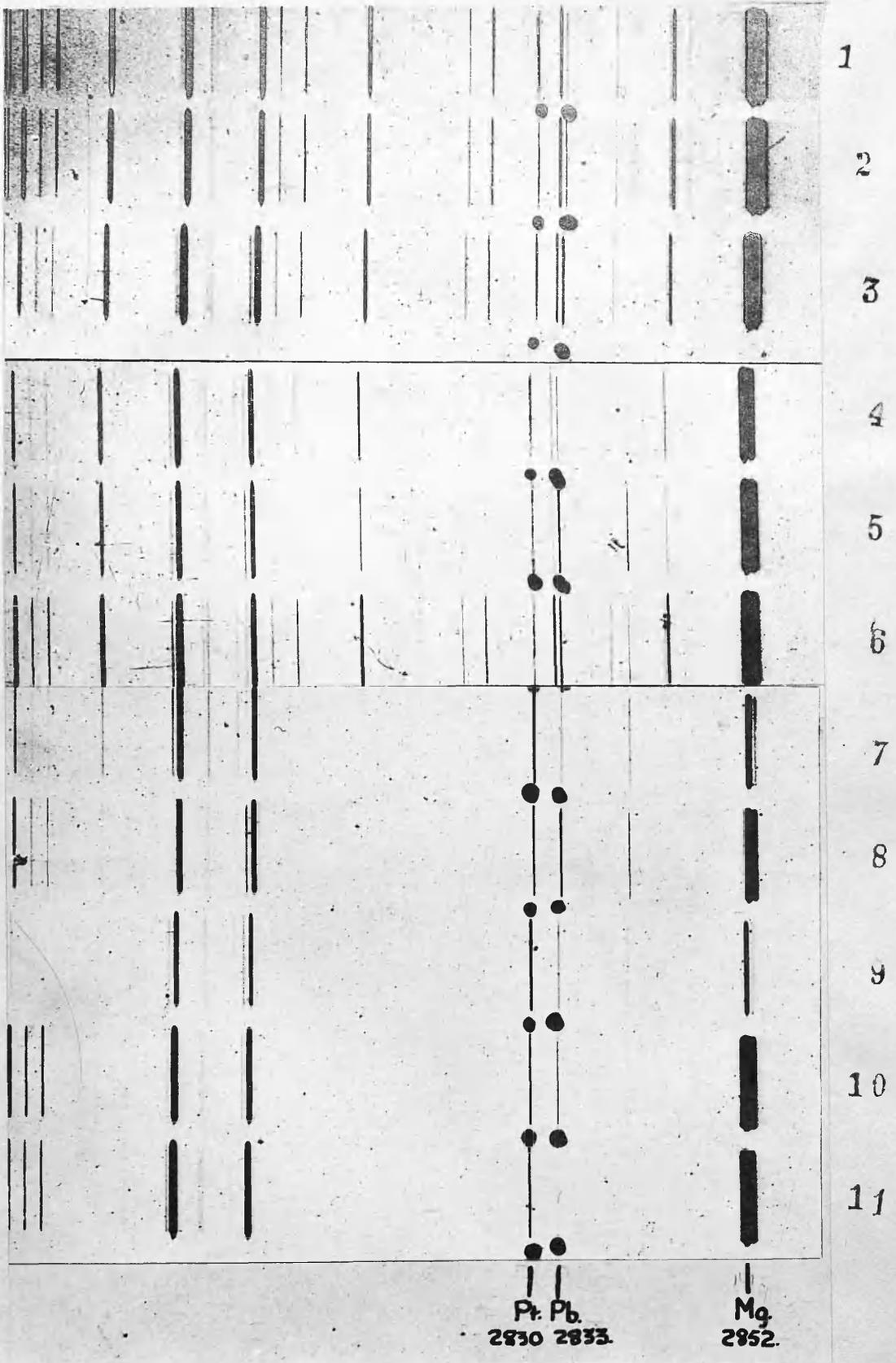
that of the adult. In this connection, while little can be concluded from a single analysis, it is of interest to note that in one pair of adult twins investigated, the blood and urine concentrations were very similar; as far as I could ascertain the exposures to lead had been practically identical from birth, (illnesses, home, diet, occupation, etc.)

TABLE III.

<u>Subject</u>	<u>Age</u>	<u>Occupation</u>	<u>Blood lead in microgms. per 100 c.cs.</u>	<u>Urine lead in microgms. per 100 c.cs.</u>
Charlie	17 yrs.	Machine operator.	17	11
Jimmy	17 "	" "	13	7

It is difficult to procure for investigation adults who have had such similar conditions of lead exposure. From these results it is evident that the infant receives lead from the maternal circulation and retains it, such lead as is excreted in the urine can be accounted for by the amount present in the milk, which is subsequently absorbed from the infant's alimentary tract. Since the lead content of the milk is governed by the lead content of the maternal circulation, and the lead content of the infant circulation is closely approximate to that of the mother's, from early intra-uterine life, and for several months

thereafter, lead metabolism in the infant is governed directly by the maternal concentration in blood and milk. There is, therefore, adequate evidence to support the theory that there is a hereditary susceptibility to lead poisoning; the manner in which the susceptibility is produced is not within our knowledge at present. Most workers are agreed that lead is more toxic to the young than to the old. (Legge, Goadby, Oliver, Croll, Nye et alii), yet at the most susceptible period of its life, the infant has in many cases a blood concentration higher than that of certain adults. This no doubt explains the fairly wide range of tolerance to increases of lead in the circulation generally found, and is another factor of hereditary origin. It is doubtful if there is any evidence of an acquired immunity to the effects of lead compounds in the body as postulated by Legge, this presupposes that lead is a toxic "occupational foreigner". From its unfailing transmission to the foetus, its retention by the foetus, and its presence in tissues and body fluids in health, I have come to the conclusion that it is a "physiological trace element", with a definite, but as yet undiscovered, function.



Magnified X 6

Mother - Infant Investigation. Section I. Part 2.

Spectrograms 1, 3 and 5 are those of maternal blood, and 2, 4 and 6 those of their respective infants at birth. The transmission of lead to the foetus is clearly indicated and it is further seen that the metallic spectra of both maternal and infant bloods are strikingly similar.

Spectrograms 7 and 9 are those of infant urines, 8 and 10 those of maternal milk collected on the 5th day. The low lead content of these fluids is evident from the weakness of their lead lines 2833 A° compared with the Platinum line 2830 A° . Note also the number of lines which have disappeared from these latter spectrograms compared with blood. This is mainly due to reduction in the number of Iron lines denoting the low Iron content in milk and urine. In blood spectrograms the line between 2830 A° and 2833 A° is an Iron line which has disappeared from milk and urine. Note also the diminution of the Magnesium content of urine, compared with milk.

Spectrogram 11 is a milk with practically no lead present. (See text 'a' and 'b' lines, page 69.)

Magnified x 10.

SECTION II.ABSORPTION OF LEAD.

In Section I, it was concluded that the blood lead concentration of the infant was maintained principally by the available lead from its main source of diet, the milk. In the adult other sources of lead besides those of dietetic origin have to be considered, principally the factors arising from social habitat, and occupation. Kehoe, ⁽⁵³⁾(1933), in his investigations found that as compared with primitive Mexican Indians, average American town dwellers have a high blood lead level; one of the factors contributory to this, he concluded, was the inhalation of atmospheric lead dust, evolved as a result of industrial processes. In general, town dwellers in highly industrialized zones, show a higher blood lead concentration than do their fellows who live in the country. Legge, ⁽⁵⁴⁾(1934), commenting on the presence of the metal in the urine of persons unexposed to lead, came to the conclusion that it originated in inhaled lead dust. A more specific source of lead absorption is that pertaining to occupation, and from this source, lead may gain ingress to the circulation by three main routes, by the

respiratory tract, the gastro-intestinal tract, and possibly by the skin.

INHALATION OF LEAD FUME AND DUST.

No doubt now exists as to the toxic effects of lead when inhaled in the form of fumes or dust, (55) although Melliere made exhaustive attempts to prove that inhalation was not a serious source of lead absorption. (56) Tanquerel des Planches, (1839), produced the symptoms of lead poisoning in experimental animals by blowing lead dust through a tracheotomy opening. (57) Legge and Goadby, (1912), confirmed this in a prolonged investigation on cats. It has been largely ignored, that these original investigators were working at a time when it was erroneously supposed that lead poisoning was acquired exclusively by absorption from the alimentary tract, and were endeavouring to acquaint the profession with a new, and hitherto unsuspected source of lead poisoning of a grave type.

Industrially, the result has been unfortunate in that lead poisoning by inhalation has been considerably over-emphasized, and absorption by the alimentary route so ignored that some authorities doubt its causal role in the production of industrial lead poisoning.

Kehoe seems to be considerably misquoted in America,
(58)
thus Johnstone, (1940), quotes him as his authority for
saying "that the bulk, if not all the ingested lead
is unabsorbed, and is excreted unchanged in the faeces".
Kehoe, in his original publication, quoted the results of
his experiments on lead ingestion with medical students
as subjects. He found that the daily ingestion of as
little as half a milligramme of lead was sufficient to
cause a detectable rise in the blood lead concentration.

A distinction should be made between inhalation
of lead fumes, and lead dust; the former as a source
of lead poisoning should be rarely encountered nowadays,
and will usually be met with, where large quantities of
material of a high lead content are burned in ignorance
of the nature of their composition, e.g., burning for
fuel of disused lead storage battery boxes, (vide
(59) (60)
Blackman's cases, (1937), McNally's case, (1943).)

It is possible that in the future, owing to the demands
of industry, and the scarcity of metal, increased
smelting of scrap may again bring this form of lead
poisoning into prominence by reason of the large
number of lead contaminated processes through which the
job has passed after casting, (boring and tapping, painting, etc.)

lead, however, does not volatilize until a temperature of 1,500°C. has been reached, and in lead processes a fume risk should not be encountered except under the protection of strict precautionary conditions.

Inhalation of lead dust is another matter, and industrially of numerous sources of origin. For such inhaled lead dust to be absorbed, it has been considered that it has to reach the alveolar spaces of the lung, where under phagocytosis, and chemical interaction with the tissue fluids in the presence of carbonic acid gas, it passes into solution, and is absorbed, with possibly a prior change to the colloidal state (Legge). This necessarily gives rise to consideration of such factors as:-

- (I) The particle size of the lead dust so inhaled.
- (II) The action thereon of the cilia of the tracheal and bronchial mucosa.
- (III) The fate of such inhaled dust, subsequently coughed up or trapped in the naso-pharynx.

From our knowledge of silicosis it is now known that only a very small proportion of sand dust particles attain the small dimensions necessary to reach the alveolar spaces (below five microns). In comparing the industrial processes entailing a risk of silicosis, and

lead dust inhalation it is unlikely that many of the lead dust particles will be as small as the silicon particle, which by its minuteness escapes the trapping action of the bronchial secretions, and the action of the ciliated epithelium lining the trachea. The vast majority of such lead particles inhaled will be swept back by the action of the cilia, subsequently coughed up, and swallowed. ⁽⁶¹⁾ Legge assumes that if particles of carbon dust are so readily demonstrable post-mortem in the lungs, there is no need to consider the fate of lead dust particles to be otherwise.

Against this, such carbon dust is readily evident to the naked eye on post-mortem examination. It would be anticipated that the typical lead lesion, capillary haemorrhages, would be readily demonstrable in the lungs at the site of entrance of the lead particles; post-mortem descriptions do not emphasize the appearance of such lesions in the lungs, even in cases of lead fume poisoning. Further, lung tissue analysed spectrographically, and chemically, does not give a result indicative of the presence of much lead in lung tissue; such lead as is present could be more than accounted for by the blood content persisting in the

tissues.

Legge, in attempting to explain the mode of lead absorption from the lungs, seeks the explanation in Armit's work on Nickel Carbonyl Poisoning, ⁽⁶²⁾ (1908) where it was found that the nickel was deposited in a finely dispersed metallic state over a considerable part of the absorbing surface of the lung. Legge did not take into consideration that the nickel so deposited, enters the respiratory tract in combination with a true gas (CO.), and that no such gaseous combination is known in the case of lead. Further, the lesions in Nickel Carbonyl Poisoning are mainly local in character, nor is there any evidence that the deposited nickel is absorbed. The picture here is very similar to that found in Metal Fume Fever (Zinc) where ⁽⁶³⁾ Drinker (1932) found that the symptoms were probably anaphylactic in origin, owing to absorption of the products of tissues necrosed under the action of the zinc. The low content of lung tissue post-mortem can only be explained by assuming that all the lead which reaches the alveolar spaces of the lungs is absorbed rapidly into the circulation before it produces any local effects; that it is small in quantity makes no difference owing to the small

amounts necessary to produce toxic symptoms, such an explanation is, however, unlikely.

The production of toxic effects by lead inhalation cannot, in our opinion, be confined solely to absorption from the alveolar spaces of the lungs. The larger particles trapped by the bronchial secretions are coughed up, and subsequently swallowed, after which absorption takes place from the alimentary tract; a similar fate overtakes the larger particles deposited in the naso-pharynx. We must regard lead poisoning by dust inhalation, therefore, as a combination of local absorption through the alveoli, and remote absorption by the alimentary tract.* Weyrauch and Schmidt (1933)⁽⁶⁴⁾ ligated the oesophagus in a group of rabbits, and exposed them along with a control group to the effects of lead dust inhalation; they found that the group with the oesophagus unligated had higher blood lead levels than the other group - the only source of such an increased blood lead could be derived from lead coughed up, swallowed, and absorbed from the alimentary tract. To these findings must be added the work of Blumgart (1923)⁽⁶⁶⁾, experimenting with lead dust on dogs, in which the trachea and oesophagus had been

* Cholak (1943)⁽⁶⁵⁾ confirms this to be the fate of inhaled lead dust.

ligated - the animals developed lead poisoning, and in a few cases died; he concluded therefore from this that lead was absorbed from the naso-pharynx. From this we are of the opinion that Industrial Lead Poisoning by inhalation of dust, has in its production, ancillary absorption from the gastro-intestinal tract and naso-pharynx. (67) Legge states that an amount of lead inhaled will produce the same toxic effects as a hundred times the same amounts of lead ingested. While pointing out that only the smaller particles of lead dust would reach the alveolar spaces, he does not comment on the possibility of his cats swallowing dust which had been coughed up, and we believe that herein Legge is in error. From the experiments of Weyrauch and Blumgart, it is probable that the amount of lead coughed up from the respiratory passages, and subsequently absorbed from the intestines, is sufficient to play an adjuvant part along with absorption from the naso-pharynx, in the production of what has hitherto been regarded as poisoning exclusively by inhalation.

ALIMENTARY ABSORPTION OF LEAD.

Lead poisoning by the absorption of lead after swallowing, is of frequent description in medical literature. (68) Tronchin (1758) described the colic arising

from the swallowing of lead contaminated wines.

(69)

John Hunter (1788) described the "dry belly-ache"

in members of the Jamaica garrison from the lead content of rum and certain other local wines. More

(70)

recently the investigations of Croll (1929) and

(71)

Nye (1929) into the high frequency of Chronic Nephritis in young children showed that Chronic Lead Poisoning by the alimentary route is readily accomplished.

In view of these findings it is surprising that

Alimentary Lead Poisoning should be so under-emphasized as a source of Industrial Poisoning, and dismissed

(72)

lightly by certain authors. Magnuson and Raulston (1940)

report the incidence of lead poisoning in roofers who

were in the habit of holding in their mouths, while at

(73)

work, nails containing .9% to 4.7% of lead. Johnstone,

in discussing this case said that it was included

(in his book) not to refute the work of Kehoe, but to

present another possible angle to the problem of

Industrial Lead Poisoning.

The chemical form in which lead is absorbed

(74)

from the alimentary tract is obscure. Legge (1912)

states that the bulk of the lead is converted into lead chloride in the stomach, but considers that no lead is

(75)
absorbed from the stomach. Weyrauch and Schmidt (1933)
on the other hand consider that some absorption from
the stomach takes place. The chloride, peptonates, and
albuminates of lead found in the stomach are passed
on into the small intestine, where sulphide of lead
is formed by combination with the sulphur set free
from the breakdown of protein in the course of
digestion. This formation of insoluble lead sulphide
is increased the more freely as the intestinal contents
pass on, and the amount of sulphur liberated by protein
digestion is increased; for this reason it has been
considered that the amount of lead capable of absorption
from the alimentary tract by reason of its insoluble
form is very small; Legge has shown that bile has the
power of solution of lead in vitro, and it is probable,
therefore, that most of the absorption takes place in
the upper part of the small intestine.

In considering the alimentary route as a source
of Industrial Lead Poisoning, the following experiment
was carried out. Four subjects, two males and two females,
were selected, and under standard conditions five cubic
centimetres of blood, serum, and urine were collected.

The subjects were then given three grain pills of lead acetate containing about 70% of lead to swallow as follows:-

- 1st day ... 1 pill.
- 2nd day ... 2 pills.
- 3rd day ... 3 pills.

On the fourth day, another set of samples was collected and spectrographed under standard conditions. The results are shown below.

TABLE IV.

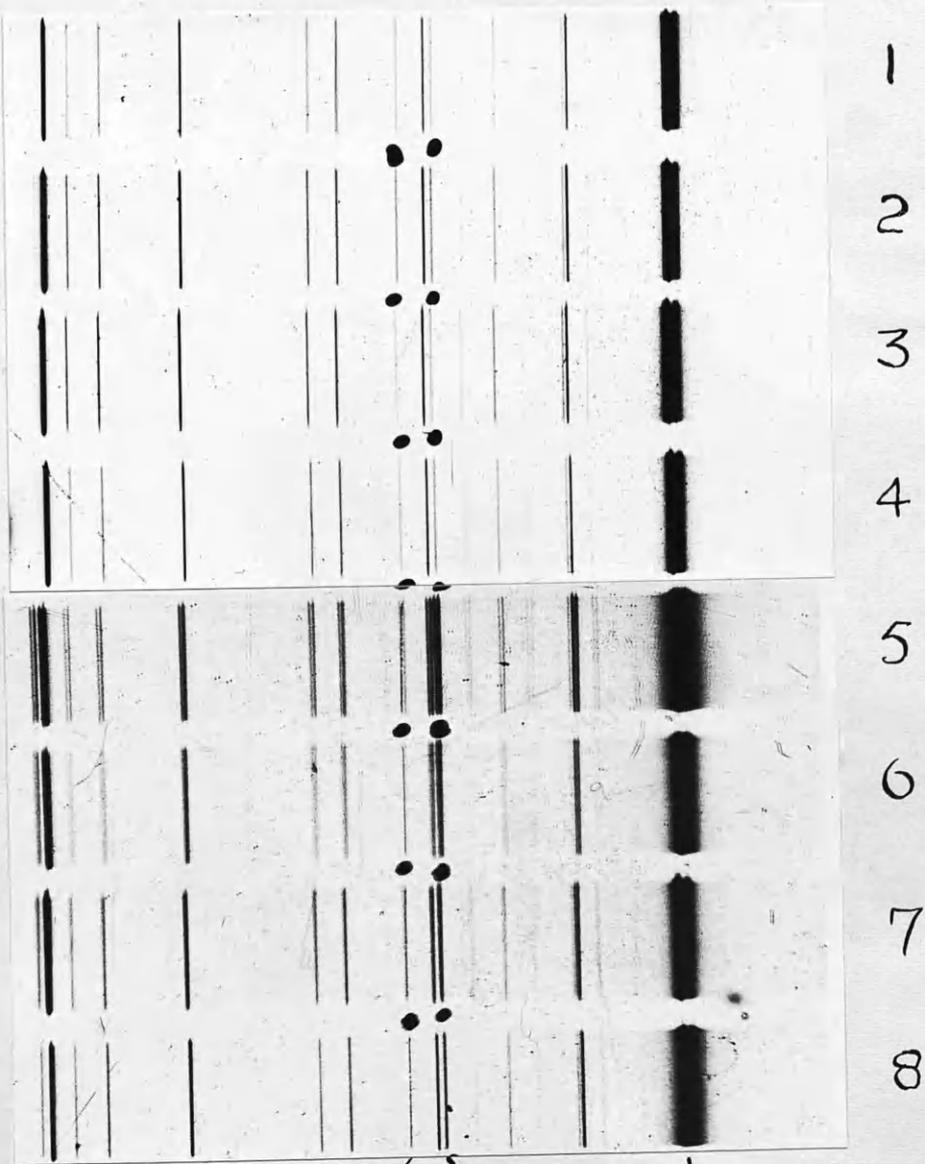
Before lead ingestion.

	Sample.	Ash weight after sulphation.	Lead content in microgms/100 c.cs.
<u>Blood.</u>	Dr. E. 5 c.cs.	.0640 grammes.	11
	Dr. T. 5 "	.0590 "	14
	Mrs. McE. 5 "	.0624 "	21
	S.B. 5 "	.0653 "	23
<u>Serum.</u>	Dr. E. 5 "	.0393 "	34
	Dr. T. 5 "	.0140 "	5
	Mrs. McE. 5 "	.0320 "	66
	S.B. 5 "	.0414 "	40
<u>Urine.</u>	Dr. E. 5 "	.1086 "	5
	Dr. T. 5 "	.0819 "	3
	Mrs. McE. 5 "	.1500 "	5
	S.B. 5 "	.1054 "	5

After Lead ingestion.

<u>Blood.</u>	Dr. E. 5 c.cs.	.0670 grammes.	250
	Dr. T. 5 "	.0620 "	155
	Mrs. McE. 5 "	.0580 "	49
	S.B. 5 "	.0800 "	58
<u>Serum.</u>	Dr. E. 5 "	.0480 "	15
	Dr. T. 5 "	.0300 "	7
	Mrs. McE. 5 "	.0420 "	200*
	S.B. 5 "	.0436 "	12
<u>Urine.</u>	Dr. E. 5 "	.0500 "	107
	Dr. T. 5 "	.0618 "	72
	Mrs. McE. 5 "	.1346 "	45
	S.B. 5 "	.0944 "	26

* Inaccuracy owing to haemolysis invalidates this result.



Pt2830 Pb2833 Mg2852

Magnified x 6.

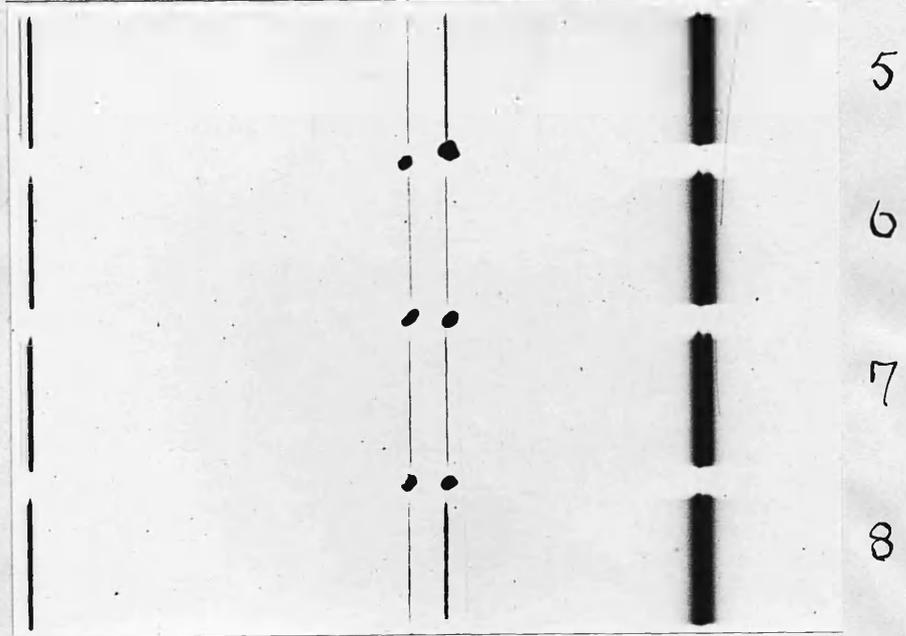
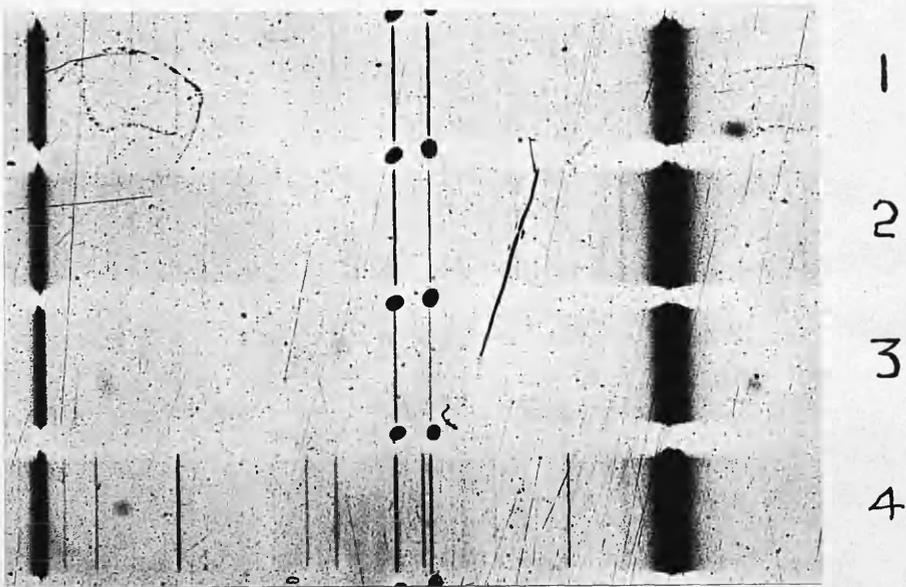
Spectrograms of Blood and Urine from the Investigation
on Alimentary Absorption of Lead.

Spectrograms 1, 2, 3 and 4 are those of blood from subjects, E., T., Mac.E., and B., before lead ingestion.

5, 6, 7 and 8 are blood spectrograms after lead ingestion from the same subjects respectively. The increase in the blood lead concentration due to absorption of lead from the intestine is particularly well shown in spectrograms 5 and 6, as compared with spectrograms 1 and 2. In spectrogram 5 the lead line has become so dense that it is tending to overshadow the Iron line beside it. (Section II Part 2. Table IV.)

The choice of the acetate of lead may be criticised on the grounds that it is one of the most soluble of lead salts, but it was useful in that it could be administered in pharmacopaeial doses. None of the subjects complained of any symptoms, nor in the case of two subjects (A.T. and M.McE.) did haemaglobin levels or blood pictures show any abnormality; it should be noted that the lead administration was based on official doses.

From these results it is clear that however insoluble may be the compounds of lead found in the gastro-intestinal tract, lead is absorbable from the alimentary tract to an extent capable of elevating the blood lead fifteen to twenty times; at first sight this is difficult to reconcile with the known insoluble properties of lead sulphide. A clear distinction is however necessary between Chemical and Physiological Solubility. In the case of the "trace elements" we are dealing with quantities of the order of millionths of a gram, and what may be extreme Chemical Insolubility, can, at the same time, constitute high Physiological Solubility. It is only on this basis that the presence in the blood stream of such metals as nickel and vanadium can be explained.



Pt 2830 Pb 2833, Mg 2852

Magnified x. 6-

Spectrograms of Blood and Urine from the Investigation
on Alimentary Absorption of Lead.

Spectrograms 1, 2, 3 and 4 are those of urine from the same four subjects before ingestion of lead.

Spectrograms 5, 6, 7 and 8 are the urine spectrograms after lead ingestion from the same four subjects respectively. The increased excretion of lead which occurs on the blood lead level rising, is clearly indicated on comparison of the spectrograms. This again is best indicated by spectrograms 5 and 6 as compared with spectrograms 1 and 2 in which the platinum line is obviously more dense than the lead line, but in 5 and 6 the lead line is of much heavier density than the platinum line.

(See results, Section II Part 2. Table IV.)

While in the experiment outlined little more than one twentieth of the total lead was probably absorbed in three days, in terms of microgrammes it is somewhere about twenty times the amount normally present in the whole circulation. I fail to see, therefore, the grounds on which Widdowson and McCance (1943) state that "there are no special characteristics in regard to elimination and absorption of the "trace elements" except in so far as can be deduced from their chemical characteristics".

These results were also confirmatory of the conclusions arrived at from the investigation in Section I, (Table II, Part 2) namely, that by exposure to relatively high lead concentrations at a susceptible period of life, the average individual has a wide range of tolerance to the effects of lead; further this tolerance could not be classed as arising from an acquired immunity to lead, as none of the subjects of the experiment had ever been exposed to a lead process. From what has hitherto been said, concerning the adjuvant role played by alimentary absorption to the absorption of lead by inhalation, the experiment, in my opinion, clearly indicates that every preventative measure against

alimentary absorption of lead should be taken, and that industrial poisoning by this route is a real danger on which too little stress is laid at present. There is wisdom in the Statutory Order concerning washing facilities for lead workers, and forbidding of the taking of meals in rooms where lead processes are carried on.

CUTANEOUS ABSORPTION OF LEAD.

Industrially, lead poisoning by absorption through the unbroken skin does not seem to be of importance. Legge pointed out, that no evidence of excessive lead absorption was evident, when he investigated a large number of men employed in the handling of considerable quantities of oleate of lead; he concluded that absorption of lead by the unbroken skin was of no industrial importance. Carnet (1825)⁽⁷⁷⁾, Manouvrier (1873)⁽⁷⁸⁾ and Druet (1875)⁽⁷⁹⁾, experimenting on rabbits with lead acetate plasters, found evidence of lead absorption; to a certain extent these experiments are similar to what is already known of the effects of wet dressings (carboloria, etc.), and cannot be considered as in any way reproducing industrial conditions, or processes. Bartlemann and Dukes⁽⁸⁰⁾ report the case of an actress

who developed lead poisoning, from the use of a grease paint containing 40% of lead; it was considered that susceptibility to lead determined the incidence of poisoning in this case, as although several other members of the cast complained of not feeling well, no definite evidence of lead absorption was found. It was not considered worth while to investigate this source of lead absorption as it is seldom reproduced industrially - therapeutically, lead applications to the skin have gone out of vogue. It is noted, however, for interest, and pertinent to later discussion on investigation of lead excretion by the skin.

SECTION III.

EXCRETION OF LEAD IN RELATION TO DIAGNOSIS.

Considering the routes by which lead gains access to the circulation, it is seen to follow closely the metabolic essentials; as regards excretion it would be expected that the elimination of lead would take place by the same channels as the by-products of metabolism, namely, the lungs, the intestines, kidney and possibly the skin.

It is probable that some excretion of absorbed lead takes place by the sputum, but proof of this cannot be obtained owing to the sources of contamination

in the mouth and naso-pharynx. Excretion by the kidney is now considered to be of more importance in the elimination of absorbed lead, than the intestine which earlier investigators considered to be the principle means of lead excretion.

EXCRETION OF LEAD BY THE INTESTINE.

(81)

Legge, (1912) in considering the absorption and excretion of lead, states that lead absorbed from the small intestine is re-excreted into the large intestine; in proof of this he instances the marked staining in the region of the ileo-caecal valve in his experimental animals; it is not clear from his description, however, in which layer of the intestinal wall this staining was encountered. Staining of the mucous layer could well arise from surface contact with unabsorbed lead; the presence of lead in the submucous layers of the intestine, could arise from absorption as easily as excretion. Some reliance for diagnostic purposes has been placed, however, on the quantity of lead in the faeces. Kehoe aptly remarks that as far as lead is concerned, one need only consider such lead as has been absorbed. All authors are agreed that a certain amount of lead is unabsorbed, and excreted in the faeces, for this reason

analysis of faeces cannot provide any indication as to what degree of absorption has taken place; all that could be concluded from a high faecal lead content, would be that there appeared to have recently been some abnormal exposure to lead. This, however, (82) does not necessarily hold good, Tompsett (1939) has shown that a high calcium content of the diet reduces the absorption of lead, so that with a high calcium diet a high faecal lead results. For these reasons I consider that analysis of the lead content of the faeces, is of little or no value for diagnostic criteria, and prefer to rely on analysis of the urine.

EXCRETION OF LEAD BY THE KIDNEYS.

The majority of investigators express the result of urinary analysis in microgrammes per litre of the total daily output, or in terms of the total daily excretion of lead in the urine. So many factors enter into possible fluctuation of the blood lead concentration, and in turn the urinary concentration, that I prefer half an hour after blood collection, to collect five cubic centimetres of urine for correlation with the

blood content; in this way definite information is obtained as to the power of the individual to excrete lead - a factor of importance where high blood concentration is encountered.

There is some diversity of opinion as to the normal maximum concentration of lead in urine. Kehoe ⁽⁸³⁾ (1933) found the average concentration to be twenty to eighty microgrammes of lead per litre of urine, with a normal maximum concentration of one hundred microgrammes per litre. Anything in excess of this latter figure he considered as being indicative of lead poisoning. ⁽⁸⁴⁾ Beaumont (1936) considers that the normal range is from one hundred to three hundred microgrammes per litre; Legge ⁽⁸⁵⁾ (1934) found that for those unexposed to a lead risk, forty-eight microgrammes per litre was the average concentration; for lead workers not exhibiting toxic symptoms, two hundred and eighty microgrammes per litre. The figures of these two British workers correspond more closely with my own findings than do Kehoe's, although racial idiosyncrasy may contribute to the difference. I have found thirty microgrammes to three hundred microgrammes per litre the range of results recurring with the greatest frequency. On

occasion, however, I have encountered results much higher, (1,500 microgrammes per litre) and so low on the other hand that the lead line at 2833 A⁰, while visible, was so faint as to be almost immeasurable. From the diagnostic aspect, one feature of importance is the fluctuation which may occur in the lead content of urine throughout the day. For this reason single samples of urine are useless for diagnostic purposes, and a source of diagnostic error, unless correlated with a blood analysis at the same time.

TABLE V.

<u>Subject.</u>	<u>Lead content in Microgms/100 c.cs. Urine.</u>	<u>Subject.</u>	<u>Lead content in Microgms/100 c.es. Urine.</u>
A.T.		J.McP.	
12.30 p.m.	44	12.30 p.m.	15.5
1.30 p.m.	20	1.30 p.m.	14.0
5 p.m.	8	5 p.m.	16

(86)

Kehoe's low figures may be due, as Blumberg (1935) points out, to the not inconsiderable loss of material which arises in the course of chemical analysis.

(87)

Barnes (1939) has suggested, that analysis of three or four hour samples of urine, are adequate for accurate

results, when the factors of specific gravity, acidity, etc., are taken into account. In our experience we cannot agree with him.

Excretion through the skin.

(88)

Serafini (1884) claimed that by means of electrolytic baths, the elimination of lead could be induced through the skin, presumably under the action of the electric current; less ingenuous investigators dismissed the source of lead found in the water, as being due to mechanical contact with the apparatus used, or contamination due to the normal lead content of the water itself. While Manouvrier and others investigated the possibility of lead absorption through the skin, as a source of excretion, the skin has been largely ignored; the question was investigated in the following manner. The two female subjects of the previous experiment after the axillae had been shaved, and cleansed with lead free alcohol, were placed in a hot room. In the axillae was placed a small quantity of Dextrosol "D" Glucose (lead-free), so that by its hygroscopic nature the sugar would absorb the sweat secreted. After two hours the samples were collected, and spectrographed under standard conditions,

with an amount of sugar equal to that which was originally used for the collection.

A marked increase in the density of the lead line at 2833 A^o. was observed in the "sweated" sample, indicating the presence of lead in the sweat. This method of investigation proved difficult, the weight of ash obtained being awkward to handle spectrographically; further, one of the subjects next day complained of sugar itch. The experiment was repeated on the other subject, using No. 41 Whatman filter papers as the vehicle for sweat collection; the temperature of the room was raised to 86^oF., and profuse diaphoresis induced by means of hot drinks, and exercise. The result obtained on the developed plate was strongly confirmatory of the result of the first experiment. Some difficulty was experienced with this method, as on addition of the sulphuric acid to the filter paper, brisk chemical reaction occurs tending to loss of material. The filter papers contain a trace of lead, and in several other subjects who were subjected to sweat collection without artificially producing diaphoresis, I did not obtain such an increase of density of the lead lines, as would

justify definite conclusions as to the excretion of lead by the sweat under normal conditions. The experiment was therefore carried further, by raising the blood concentration by lead ingestion and inducing diaphoresis; the result showed that lead was present in the sweated samples, conclusively proven by the presence of "b" lines in the sweated filter paper ash, while the "a" lines only appeared in the control filter papers.

It would seem therefore that lead is excreted in the sweat in very low amounts under normal circumstances, but on increase of the blood lead, the concentration in the sweat increases also. The amount of lead in the sweat cannot be estimated, as the specific gravity of the sweat varies from case to case, and the weak base spectrum would probably cause quantitative analytical errors. The result is of importance in contributing to our knowledge of one of the reasons for fluctuation in the blood and urine concentration, and is worthy of consideration from the aspect of treatment. Spontaneous toxic episodes, or acute symptoms during de-leading, would be more adequately controlled by treating the case on the same lines as an acute nephritis, the high concentration of lead in the circulation being more rapidly reduced by

diuresis, and diaphoresis. From time to time Balance Experiments are reported, in which the difference between the amount of lead absorbed, and that excreted is taken as indicative of the lead stored in the tissues; in view of the excretion of lead in the sweat, which cannot be estimated, such balance studies on lead storage in the tissues are valueless.

Excretion of Lead in Bile.

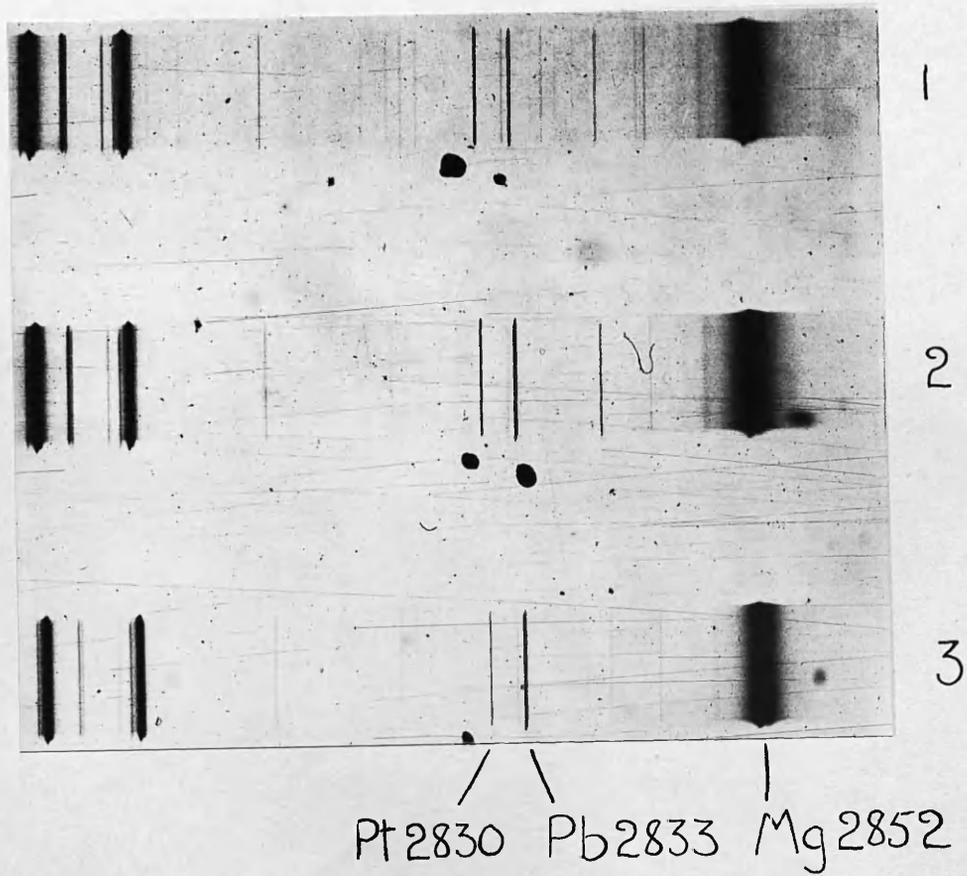
(89)
Steinberg (1910) suggested that lead was excreted
(90)
in the bile, and Reznikoff (1940) supports this view. Legge commenting on Steinberg's hypothesis pointed out that unless the bile is excreted from the liver in an insoluble form, there is nothing to prevent its reabsorption from the small intestine setting up a constant cycle of lead absorption. He considered that excretion of lead in bile should not be accepted without adequate experimental investigation. In the human subject for such investigations, reliance has to be placed on post-mortem material; I have not had the opportunity of analysis of a case of liver bile from a subject with biliary fistula. The results obtained cannot therefore be regarded as "normal", and the factors

of concentration of bile by the gall bladder, and the terminal illness of the subject have to be taken into account.

The samples of bile were collected by aspiration of the bile from the gall bladder into a syringe, measuring five cubic centimetres, and transferring to a silicon crucible.

TABLE VI.

<u>Sample.</u>	<u>Cause of death.</u>	<u>Lead content Microgrammes/100ccs.</u>
5ccs.Bile.	Subacute Bacterial Endocarditis	48
5ccs.Bile.	Chronic Cardiac Failure.	27
5ccs.Bile.	Cerebral Haemorrhage.	31



Spectrograms of Bile from gall bladder.

The presence of lead in the bile is proved by the definite lead lines present in the three samples analysed . Some reduction of Iron is also present in bile when compared with blood as is common to all the other tissue fluids.

From these results it is clear that lead is excreted in the bile, and assuming that the gall bladder concentration is ten times that of liver bile, the lead content of the latter may be assumed to be approximately one tenth that of average blood, or rather less than the lead concentration of urine. There is however no grounds for the assumption by certain workers, that the bulk of the lead absorbed from the intestines, does not reach the systemic circulation by reason of its excretion from the liver.

The reabsorption of lead excreted in the bile is difficult to understand, yet there are no real grounds for denying that it happens. Legge in his experiments on cats, and artificial lead digestion in the laboratory, found that lead was dissolved by the intestinal juices to a considerably less degree, when subjected to pancreatic digestion, without previous digestion in the stomach. It is possible that the explanation of reabsorption from the bile lies, at least in part, in these results reported by Legge.

EXCRETION IN HAIR AND FINGER NAILS.

Meilliere was the first to draw attention to the presence in the human hair of lead, but in my

investigation I cannot confirm that the amount present is large. The samples were first completely immersed in absolute alcohol and then washed in Ether. This is necessary to get rid of surface contamination.

In the hair most of the elements present are the same as those in the blood, though markedly reduced in quantity with the exception of zinc, nickel and silver. In one of the subjects a very dense nickel spectrum was found. Hitherto consideration of this source of metallic excretion has been confined to arsenic, but it is common to all the metallic trace elements. A similar spectrum was obtained from spectrograms of the nails, which showed rather a higher metallic content than the hair. Strong lines of copper, lead, and silver, however, I regarded as indicative of surface contamination, e.g., in the finger nails, to say nothing of occupation, the handling of coins of silver and copper would provide a certain source of contamination. While it is difficult to assess how much surface contamination contributes to the spectrogram obtained for nails, I am of the opinion that in the case of the hair, most of the contamination was got rid of by washing in ether.

LEAD CONTENT OF THE DIET.

Just as the infant's blood lead is maintained by the absorption of lead of dietary origin, so in the adult, the foodstuffs ingested provide a contributory source to the main occupational factor. Gautier (1881) seems to have been the first to comment on this source of lead absorption; he estimated that the average Frenchman absorbed more than half a milligramme of lead daily in his diet, and attributed it mainly to contamination in foodstuffs. He was probably the originator of the expression "normal lead", which began to appear in French literature about the beginning of the present century. These French workers, however, do not seem to have appreciated that all natural foodstuffs contain lead in variable quantities; the amount of lead ingested in the diet considerably exceeds half a milligramme, but what proportion of this is absorbed, is a matter of conjecture.

Water has generally been credited with being the main source of lead in the diet. Glasgow water evaporated from a volume of one gallon to dryness gives a result equivalent to nine microgrammes per hundred cubic centimetres;

in the table below is the lead content of some of the commoner foodstuffs in our diet today.

TABLE VII.FOODSTUFFS.

	<u>Substance</u>	<u>Ash wgt.</u>	<u>Pb.</u>	<u>Pt.</u>	<u>Log/R</u>	<u>Micro/100 gms.</u>
5 grammes	Sugar	.0064	4.7	3.1	.18	5
" "	Leek	.0530	5.6	10.1	T .74	43
" "	Bread	.1320	3.3	12.8	T .41	168
" "	Cheese	.2626	8.2	15.1	T .74	43
" "	Sausage	.1832	6.1	17.4	T .55	85
" "	Potato (Peeled)	.0586	5.4	10.5	T .71	37
" "	Carrot	.0362	7.6	14.1	T .73	42
" "	Biscuit	.1234	2.7	12.6	T .35	216
" "	Tea(Dry)	.3524	4.6	4.6	Zero	11
" "	Milk	.0500	10.6	16.6	T .8	28

The total lead of dietary origin available for absorption, must be fairly high when considered over the course of a day. Kehoe's (1933) findings on foodstuffs are similar, although the British and American diet is widely different. The protein foodstuffs generally show a higher lead content than the carbohydrates, and this is not unexpected in view of the animal origin of most of them. Many of the protein foodstuffs are derived from animals which feed on soils and drink stream water of variable lead content. Cattle are notorious

for licking posts, and fences which often have been painted with lead-containing paints, or containing lead in their wiring. A proportion of the animal lead must in turn be passed on to the consumer. The same applies to vegetables grown on lead soils, and beverages, although the latter probably acquire their lead contents mainly by contamination in the process of manufacture. Kehoe found a high lead content in certain American "soft drinks" of the lemonade variety. This lead content of the diet is of interest, in that taken with various other factors, it helps to explain one feature of blood lead concentrations which at first puzzled us considerably, namely, the fluctuation in the blood lead content of the individual, when identical samples are taken on different days, and even on the same day.

SECTION IV.

RANGE OF NORMAL BLOOD LEAD CONCENTRATIONS, AND FACTORS INFLUENCING VARIATIONS.

The different analytical procedures used have led to some diversity of opinion as to the normal range of blood lead concentrations; to the analytical variation, must be added those due to such factors as the "hereditary basal lead", occupation, diet and excretion.

(91)

Kehoe, Thamman and Cholak (1935) reported the normal blood concentration to range from sixty, to one hundred microgrammes per hundred cubic centimetres of blood. Tompsett and Anderson (1935) reported fifty to seventy microgrammes per hundred cubic centimetres of blood. Teisinger (J) (92) (1936) reported forty-one to seventy-nine microgrammes of lead per hundred cubic centimetres of blood on a series of fourteen cases, Taeger and Schmitt (94) (1937) reported finding concentrations of twenty to eighty microgrammes per hundred cubic centimetres of blood. All these workers were using the chemical method of analysis. Using the spectrographic method, Blumberg (95) (1935) in a large series of cases found the normal concentration to range from five to one hundred microgrammes per hundred cubic centimetres of blood. Cholak (96) (1943) found the results by the spectrographic method, to be rather lower than the original chemical investigations in which he participated; he found that thirty to eighty microgrammes was the normal range, and this is more in keeping with the work of other investigators than his original findings.

From a large series of cases investigated using the

method described, I have found that the normal range of blood lead concentration is from eleven to one hundred microgrammes of lead per hundred cubic centimetres of blood; the majority of these results fell between thirty and fifty microgrammes per hundred cubic centimetres, the mean concentration in eighty cases being forty microgrammes per hundred cubic centimetres of blood. Results as high as one hundred and eighty microgrammes per hundred cubic centimetres were encountered without symptoms, but these latter results were considered to be due to a recent temporary exposure to lead, and were not classed as normal. The results reported by various workers, while somewhat diverse as to range of normal concentrations, are fairly well agreed on the upper normal limits, and generally it will be found to be around one hundred microgrammes per hundred cubic centimetres of blood.

(97)

Willoughby and Wilkins (1938) commenting on the diversity of results from the hands of competent observers, consider that it may originate from the following factors:-

- (I) The precision of the analytical method employed.
- (II) The danger of adventitious contamination error.
- (III) Geographical Distribution.
- (IV) Influence of Disease.

The most important of these factors is the extrinsic influence on results of the method employed, and contamination error; these I have already discussed. Intrinsic factors such as geographical distribution, and disease, must also play their part in causing variation, but of considerably more importance are the factors of occupation, diet and excretion.

SECTION V.

FACTORS CAUSING FLUCTUATION IN THE BLOOD LEAD LEVEL.

OCCUPATION:

Generally individuals whose employment involves the handling, or working of metals in all their diverse forms, have higher blood lead levels than their fellow workers in offices, or sedentary occupations. The absorption and swallowing of dust, or inhalation of lead fumes, day in, day out, maintains the blood lead level in its elevated state. The following table illustrates the divergence of blood lead levels associated with employment.

TABLE VIII.

Results in Microgms. per 100 Mls.

133

Subject.	Occupation.	Blood lead microgms. per 100 mls.	Age.	Sex.
1. R.D.	Plater	47	28	M.
2. J.M.	Plater	42	27	M.
3. *W.C.	Buffer.	120	35	M.
4. *A.K.	Buffer.	150	52	M.
5. E.J.	Typist.	22	24	F.
6. S.B.	Nurse.	24	27	F.
7. J.C.	Foundryman.	42	46	M.
8. C.C.	Foundry craneman (Monel).	9	48	M.
9. B.L.	Chemist.	43	24	M.
10. L.McK.	Chemist.	50	21	M.
11. C.D.	Office worker.	14	22	F.
12. *C.A.	Labourer.	52	29	M.
13. *P.G.	Labourer.	200	48	M.
14. G.G.	Painter.	47	52	M.
15. B.D.	Foundry craneman (brass).	60	43	M.
16. *J.McD.	Machine man.	70	30	M.
17. *M.Mc.	Janitor.	56	25	M.
18. A.T.	Office worker.	15	32	M.
19. J.E.	Office worker.	11	45	M.
20. S.M.	Machine operator.	12	20	F.
21. A.W.	Dilutee welder.	12	19	M.
22. C.L.	Transport.	17	18	M.
23. W.L.	Apprentice fitter.	11	18	M.

* Subject to much dust inhalation.

(98)

This agrees with the work of Kehoe (1933)
 (99)
 and Dreessen (1943). Those who are exposed to the
 swallowing, or inhalation of dust show the highest
 blood lead levels.

In the individual this exposure may induce a
 sharp temporary rise in the blood lead level, and was
 the cause of several very high results in my
 investigation; it is comparable to the considerable
 elevation of the blood lead noted in the previous
 experiment. Sampling of blood from a group of
 individuals with a recent exposure, would tend to give
 a false picture of the normal range of blood lead
 concentrations, and is a possible factor bearing on the
 diverse results commented on by Willoughby.

DIET:

Another factor is the lead content of the diet;
 certain articles of diet (especially under war conditions)
 being of high lead content. Bread, one of the staple
 articles of diet is high in lead; in turn, sausages,
 a common article of diet both in peace time and war,
 has a heavy lead content, accentuated in war time by
 the amount of bread in them; it is immaterial whether
 the lead occurs naturally, or results from contamination

due to handling, it is there.

The diet therefore of the cases investigated by the workers mentioned is still another source of the diverse results encountered, especially when the origin of the reported figures comes from countries so diverse in their diet as Germany, America and Britain; comparison of Kehoe's table of the foodstuffs he analysed with our own, would seem to bear this out. ⁽¹⁰⁰⁾ Tompsett ⁽¹⁰¹⁾ (1941) has shown that other dietetic factors may influence lead absorption. He found that a high calcium diet diminished lead absorption, and conversely a low calcium diet increased it; this aspect of lead absorption is one of acute controversy in American literature at present.

LOSS IN SWEAT.

Any process which induces profuse diaphoresis such as Foundry casting, Heat treatment of steels, or Steam Turbine testing will cause a further variation in the blood lead of the case investigated. Foundrymen in particular, are exposed to the fumes of molten metal which all contain lead as a contaminant; yet the blood lead of these individuals is not unduly high. The profuse sweating, and diuresis, induced by the fluid drunk to make up the loss of water in the sweat, serve as an adjuvant means of keeping the blood lead below a dangerous level.

None of the previous workers have apparently attempted to repeatedly analyse the blood in a series of cases, to determine the fluctuation in the individuals blood lead level from week to week, or from day to day. The small number of cases on which I have had the opportunity to carry out repeated sampling bears out what has been said concerning the influence of occupation and diet as is shown below:-

TABLE IX.

Results in Microgrammes/100 c.cs.

<u>Subject.</u>	<u>Sampling Interval.</u>	<u>Blood Lead.</u>
A.T.)	Seven weeks.	42
A.T.)	-	14
R.B.D.)	Six weeks.	73
R.B.D.)	Six weeks.	43
R.B.D.)	-	27
J.M.)	Six weeks.	58
J.M.)		21
J.M.)		76
(102)		

Cholak (1943) carrying out repeated sampling on two individuals from week to week for purposes of comparing the accuracy of the dithizone and spectrographic method, reports results which are confirmatory of these findings. Repeated investigation in the course of a single day is difficult, owing to failure of co-operation on the part of the subjects of the investigation, but the following results show that there is a variation in the blood lead content throughout the day.

TABLE X.

<u>Subject.</u>	<u>Sample.</u>	<u>Time of Collection.</u>	<u>Result.</u>	<u>Variation.</u>	<u>*</u>
J.McD.	5ccs.Blood	10.30 am.	14	56 microgrammes=	
J.McD.	5ccs.Blood	4 pm.	70	approx.3360 micro-	
				grammes in terms	
				of whole circul-	
				atory volume.	
J.McC.	5ccs.Blood	10.30 am.	28	32 microgrammes=	
J.McC.	5ccs.Blood	4 pm.	60	approx.1920 micro-	
				grammes in terms	
				of whole circul-	
				atory volume.	

* Assumes that the total volume in the circulation is about six litres.

The influence of diet and occupation, must be the main source of variations encountered in the individual, over such a short period of sampling.

The influence of disease must play some part also in causing variation in the blood lead concentration; since this concentration is dependent on the metabolic activity of the subject, any metabolic upset will accordingly tend to cause variations in the blood lead levels; this is entirely different from considering that disease by interfering with metabolic activity, inevitably places the subject in danger from the effects of lead absorbed; the variation in most cases will be within normal limits. For this reason nevertheless, I have always endeavoured to select as subjects healthy workmen whose blood concentration will accordingly be representative of what is "normal".

Many of the results of previous workers have been obtained from patients in hospital, and the fact of their condition ignored when assessing the range of concentrations found; the effects of hospital diet, and treatment undergone, also seem to be ignored. Kehoe used normal medical students for investigation where possible, and seldom reports a result on a

hospital case, without also reporting the condition from which the case is suffering.

In considering "normal blood lead", in my opinion the only thing that matters is the upper normal maximum range of concentration; from my cases taken from healthy subjects, I consider that this is about one hundred and thirty microgrammes per hundred cubic centimetres, and symptoms will appear, and increase in severity from upwards of one hundred and fifty microgrammes per hundred cubic centimetres dependent on such factors as hereditary susceptibility, or power of excretion. Occasionally a case of lead poisoning will be encountered below this level of concentration, but probably never below a concentration of eighty microgrammes per hundred cubic centimetres in its most extreme form.

The individual fluctuation from day to day, and from week to week, is of considerable importance from the diagnostic point of view. Where reliance is placed on a single analysis of a blood sample, sooner or later serious diagnostic error will arise, by reason of the factors above mentioned; especially

when, as too often happens, the case walks in from the street to the out-patient department of the hospital for examination. This is well exemplified by the following case.

W.G. a painter came to us complaining of attacks of severe abdominal pain, associated with constipation and intermittent attacks of diarrhoea. He had no other complaint but volunteered the ominous information "that the strength was leaving his arms, and he felt he could hardly lift the brush". He was alcoholic of habit, and said that on occasion he suffered from severe headache, but had no visual disturbances. His past history revealed that he had been a painter for forty years, and in the old days often used to do "the burning", - a reference to the old practice of burning off paint from jobs after chipping off the outer coats. Examination was completely negative.

Heart.	N.A.D.
Lungs.	N.A.D.
Gastro-intestinal.	N.A.D.
Urine.	Trace of albumin.
Blood Pressure.	140/80.
Red Blood Cells.	4,600,000 per c.mms.
White Blood Cells.	6000 per c.mms.

Haemoglobin	80%.
X-ray gall bladder and stomach.	Negative.
Barium enema.	Negative.
Blue line on gums.	Absent. (Artificial teeth).
Punctate Basophilia.	Absent.

The case was diagnosed as a gastro-enteritis, but the symptoms persisted with a marked deterioration in his condition. His power of grasp was definitely normal for a man of his physique, but it was decided to investigate his blood lead concentration. The result was two hundred microgrammes per hundred cubic centimetres of blood. He was accordingly referred to hospital for confirmation of the diagnosis and treatment; he was admitted a week later, after blood estimation by the Dithizone method had revealed a concentration of one hundred and thirty microgrammes, and a diagnosis of lead poisoning made.

Careful search for a source of poisoning failed to show any recent exposure. A few days after admission to hospital, a second sample was estimated by the Dithizone method, with the surprising result of forty-four microgrammes per hundred cubic centimetres of blood.

His symptoms had cleared up and a second sample was sent to us from the hospital for analysis, - the result, fifty microgrammes of lead per hundred cubic centimetres of blood. He remained well, and has had no trouble since. This case was useful in that a check of each result was available, and both high and low concentrations were found by two different methods; a single estimation result would however have led to an inevitable diagnosis of lead poisoning. It was ultimately decided that he had not been suffering from lead poisoning by reason of the rapidity with which the symptoms cleared up, the low results of later blood examinations, and lack of clinical criteria.

It was possible that the original high blood lead was due to an incidental exposure to lead in the course of the illness, but was not in any way concerned with his incapacity. A spontaneous toxic episode would not have cleared up so rapidly.

I consider it absolutely essential that where it is necessary to rely on blood analysis in doubtful cases of poisoning, that no diagnosis be made, until at least

three analysis show a blood lead concentration of over one hundred and thirty microgrammes, and where possible, control over exposure prior to sampling should be exercised by admission of the case to the wards for investigation - not the out-patient department. There is a tendency to regard the blood lead concentration as being a fixed amount, but it is far from varying within narrow limits even in health. Probably the nearest we can get to a statement of "normal blood lead" for the individual is that indeterminable quantity, the hereditary basal lead with which we are born.

SECTION VI.

RATIO CONCENTRATION OF BLOOD LEAD AND URINE LEAD.

Kehoe was probably the first to recognize that comparison of blood and urine concentrations, might be of value in assessing departures from normal, where cases of lead poisoning were encountered. It is however difficult to assess the figures reported, owing to the lack of a standard method in applying them. Kehoe assesses the lead concentration of fifty cubic centimetres of blood, against that of a litre, of the total daily output of urine. Since the purpose underlying

such a comparison is to assess the efficiency of excretion by the kidney against a definite blood lead concentration, I prefer to correlate the concentration in blood and urine at the time of sampling. When the bladder is emptied half an hour before blood collection, urine collected about half an hour after, may be taken as representative of the renal excretion of lead, for the time at which blood was collected. To be strictly accurate, Kehoe would require to collect four hourly samples of blood, and compare the mean result of both blood, and that of the total daily output of urine. This may seem to be highly critical, but in my opinion, it is quite possible using Kehoe's method to obtain a result which is totally misleading as to the power of the kidney to excrete lead (Tables V and X). The loss of lead in the sweat under normal conditions is so low as to have an insignificant effect on these results.

(103)

Tompsett (1939) found that following lead absorption, the blood lead and urine lead concentrations did not run parallel. The results shown on Table IV. are confirmatory of these results, although in my

experiments the response of the kidneys to increased lead absorption seems to have been more prompt and vigorous than in Tompsett's cases. It is evident, nevertheless, that where the blood concentration is elevated, the kidneys attempt to balance the absorption by increased elimination. This is shown by a change in the blood/urine lead ratio from, say, 6/1 to 2/1, i.e. where lead absorption is increased, the lead concentration ratio tends to approach unity. In my experiment, in spite of the sharp increase in the blood lead, none of the experimental subjects complained of symptoms; it would appear that one of the factors determining the onset of lead poisoning, is failure of the renal excretion of lead to keep pace with increased blood concentrations.

(104) (105)
Kehoe (1940) and Gant (1940) consider that the normal blood urine ratio is ten to one, in other words, the lead concentration of one hundred cubic centimetres of blood, is equal to that of one litre of urine, although Kehoe draws attention to occasional variation both ways from this figure. Even allowing for differences in analytical and sampling procedures, I consider that a

ratio of 10/1 represents too great a difference in blood and urine lead concentration. In my experience the normal ratio is from 6/1 to 2/1 with a tendency to the lower ratios irrespective of age or occupation. The higher ratios are usually encountered in individuals with high "normal" blood leads. In the cases reported (106) by Tompsett (1939) the normal cases showed ratios varying between 3/1 and 11/1; on the other hand in cases of lead poisoning, the ratios varied between 3/1 and 40/1, - the more severe the symptoms, the greater was the divergence encountered in blood and urine concentrations.

Apart from normal cases, two types of case may be encountered industrially, the sub-clinical lead poisoning, and established lead poisoning. The former type of case is that in which the concentration ratios remain within normal limits in spite of marked elevation in the lead content of both blood and urine, and while indicative of exposure to lead absorption, and the necessity of a check on working conditions, need not be viewed with concern, as evidence of satisfactory lead elimination is present. A high blood lead with a disproportionately low urine lead on the other hand, is

a matter for concern, indicating that lead is accumulating in the blood, and tissues.

It is of interest to note that in Tompsett's cases of lead poisoning, while in many instances the blood lead was grossly elevated, the urine lead remained within normal limits. In chronic exposure to lead absorption some metabolic factor tending to lead retention must be present. As will be discussed later, most investigators are agreed that the bulk of the absorbed lead combines with the red cells, not the serum; some fixation or chemical affinity to lead must exist in the erythrocytes, but what determines this fixation, and when it occurs is unknown. It is possible that excess of lead in the blood stream leads to the formation of different metallo-proteins than occurs normally, or possibly, that excessive elimination of other metals, takes place in the presence of chronic exposure to increased lead concentrations.

SECTION VII.

PLASMA/CELL LEAD DISTRIBUTION.

Considerable diversity of results has been reported by different investigators on the distribution of lead between the cells and serum. Knowledge of the

distribution of lead between cells and serum, has been sought in an endeavour to obtain additional diagnostic criteria for the diagnosis of lead poisoning.

(107)

Aub (1926) in his cases of experimental lead poisoning, found that the bulk of the lead was carried by the plasma. Teisinger (1936) found that the results were variable, but that the plasma content was generally

(108)

higher. Tompsett (1941) similarly found that the results were variable, but in his cases the majority showed a higher lead content in the cells in the ratio

(110)

of three to one. Willoughby and Wilkins, (1936) failed to detect any lead in the plasma. Blumberg

(111)

and Scott (1935) in a series of eighteen cases of lead poisoning, found almost all of the lead in the

(112)

cells. Kehoe (1940) considers that never less than 85% of the lead is carried by the cells. In reading the work of different investigators, it is not always clear if they are applying correcting factors for volume, or allowing of correction for methods of separation.

Tompsett, while stating that a correction is made for volume, does not state what it is, and reports the ratio of whole blood/plasma as 2.8/1 - 3/1.

Tompsett took equal volumes of whole blood, one of which was centrifuged to separate the plasma, and the results are compared. Other workers (Kehoe, Teisinger et alii) separate the cells and plasma by use of Heparin and sodium citrate, so that in a given volume of blood no correcting factor is applied.

The method I chose was to draw twenty cubic centimetres of blood, of which five cubic centimetres was transferred to the crucible, the other fifteen cubic centimetres of blood was then centrifuged. While the plasma volume is about one half of that of whole blood, I found that fifteen cubic centimetres of blood was necessary to obtain five cubic centimetres of clear plasma. The results were then obtained for five cubic centimetres of plasma against an equal volume of whole blood. In my results also variability of distribution was found, in some cases all the lead must have been present in the plasma, but in the majority of cases a higher proportion was present in the cells.

TABLE XI.

Subject.	Sample.	Microgrms/Pb. per 100 c.cs.	Ratio of whole blood to plasma. (approx.)	Cell/serum ratio.
A.T.	5 c.cs. Blood.	14	(A.T.) 5/1	(A.T.) $12.5/1.5 = 8/1$.
A.T.	5 c.cs. Serum.	3		
J.E.	5 c.cs. Blood.	12	(J.E.) 1/3	(J.E.) No lead in cells.
J.E.	5 c.cs. Serum.	34		
J.B.	5 c.cs. Blood.	23	(J.B.) 1/2	(J.B.) $3/20 = 1/6$.
J.B.	5 c.cs. Serum.	40		
A.W.	5 c.cs. Blood.	12	(A.W.) 4/1	(A.W.) $10.5/1.5 = 7/1$.
A.W.	5 c.cs. Serum.	3		
C.A.	5 c.cs. Blood.	53	(C.A.) 1/1	(C.A.) $23/30 = 4/5$.
C.A.	5 c.cs. Serum.	59		
W.G.	5 c.cs. Blood.	43	(W.G.) 5/1	(W.G.) $38.5/4.5 = 9/1$.
W.G.	5 c.cs. Serum.	9		
P.A.	5 c.cs. Blood.	200	(P.A.) 5/1	(P.A.) $178/22 = 8/1$.
P.A.	5 c.cs. Serum.	44		
J.D.	5 c.cs. Blood.	70	(J.D.) 3.5/1	(J.D.) $60/10 = 6/1$.
J.D.	5 c.cs. Serum.	19		

Since approximately one half of the volume of whole blood is made up of cells, the lead content of cells per 100 c.cs. of blood = whole blood content - $\frac{\text{Plasma content}}{2}$
 e.g., (A.T.) $14 - 3/2 = 12.5$

∴ cell/plasma partition = $12.5/1.5 = 8/1$.

Since the 10ccs. of whole blood are required to give 5ccs. serum, the result obtained on 5ccs. serum is divided by two to give the serum lead content of 5ccs. whole blood.

I cannot agree with Kehoe however that never less than 85% of the lead is invariably contained by the cells, nor with Willoughby and Wilkins, that the lead content of the serum is insignificant if any be present at all. The results of the experiment on Table IV are confirmatory of the work of Blumberg and others, that in lead poisoning the bulk of the lead present is to be found in the cells. What determines this fixation of lead to the cells is not clear, but it seems definitely to take place; in three of my experimental subjects there was a definite increase of the lead content of the cells, even where previously the bulk of the lead had been present in the plasma (Table XII). In only one of Tompsett's experimental animals was the plasma content higher than that of the cells after lead absorption. In this case however the lead exposure was so gross (.5 grams. of lead acetate direct into the rabbit's stomach) that it can be ignored.

(113)
Kehoe(1942) experimenting with rabbits found that in lead poisoning, 90% of the lead was to be found in the cells; it is of interest to note that he failed to detect any difference between venous and arterial blood in this respect.

TABLE XII.

Results in Microgrammes per 100ccs.Before lead ingestion.

Subject	Sample	Result	Ratio <u>whole blood</u> serum	Ratio <u>cells</u> serum
A.T.	5ccs.Blood	14		
A.T.	5ccs.Serum	3	5/1	8/1
J.E.	5ccs.Blood	12		
J.E.	5ccs.Serum	34	1/3	No lead in cells.
J.B.	5ccs.Blood	23		
J.B.	5ccs.Serum	40	1/2	1/6

After lead ingestion.

A.T.	5ccs.Blood	155		
A.T.	5ccs.Serum	7	22/1	50/1
J.E.	5ccs.Blood	250		
J.E.	5ccs.Serum	15	16/1	33/1
J.B.	5ccs.Blood	58		
J.B.	5ccs.Serum	12	5/1	9/1

Since the serum volume is about half that of whole blood, 10ccs., whole blood are necessary to give 5ccs., serum. The serum content of 5ccs., blood is therefore obtained by dividing the result obtained on 5ccs., serum by two. This result subtracted from that of 5ccs., whole blood gives roughly the cellular lead content of 5ccs., whole blood.

Some basis for divergence of results, may be attributed to the method employed in separation of cells and plasma, and the sensitivity of the analytical method. The use of anticoagulants and Heparin always raises the possibility of contamination error. I have always avoided potassium oxalate, and sodium citrate by reason of the lead with which I have found them to be contaminated. In cases where these substances are used, it will usually be found that lack of sensitivity of the analytical method employed, necessitates such a large amount of analytical material, that estimation of plasma, and whole blood in equal volume is impractical; recourse is therefore necessary to separation of cells and plasma, in one analytical sample. I am doubtful even with the use of Heparin, that complete separation of cells, and plasma can be achieved, and the results of Kehoe, Johnstone et alii are therefore open to question. The comparative lack of sensitivity of colourimetric methods of estimation for small quantities, coupled with loss of analytical material, makes readily understandable the failure of Willoughby and Wilkins to detect lead in the serum.

Blumberg, using a spectrographic method of analysis, was faced with difficulty in measuring the line densities, and could easily fail to detect the small quantity present in the serum when it was low. Johnstone considers that no lead is normally present in ten grammes of serum (ten cubic centimetres) presumably he means an undetectable quantity. To this I can only say that I am confident that by use of the method described, I would never fail to detect lead in the serum in quantities of one cubic centimetre.

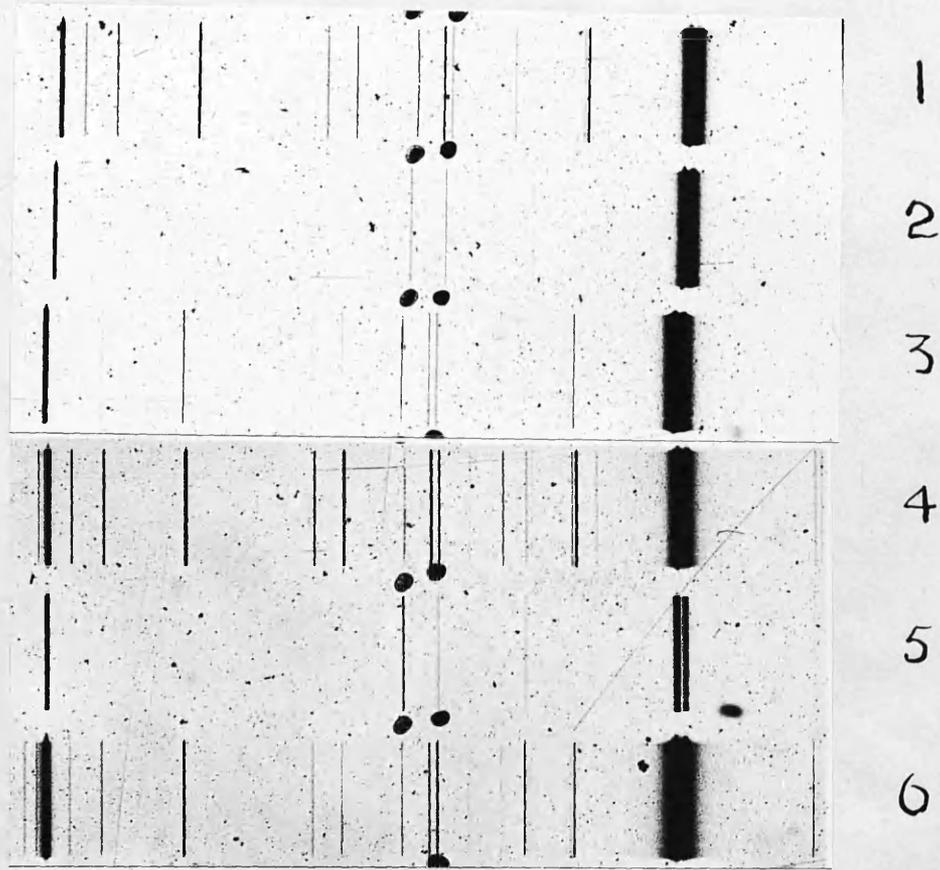
One difficulty in these estimations lies in the process of separation of cells and serum, in that no matter what care is exercised, there is always the danger of breakdown of the erythrocytes during centrifuging, with subsequent liberation of their lead content into the plasma. I have considered that the faint rosy tinge of certain plasma samples resulted from this, even where all care was taken in centrifuging. Delay in centrifuging, and exposure to variable temperatures, seems to increase the possibility of this source of error, but in many cases it will occur in the presence of the greatest care in sample collection. What the influence is on results is hard to say, but it is yet another possible factor

contributory to some of the divergent results reported. The variation in the daily lead content of urine, led me to consider that a variation in the cell/plasma distribution would similarly occur during the course of the day. While the number of subjects on which such an investigation could be carried out was low, there are strong grounds for believing that it does occur.

TABLE XIII.

Results in Microgrammes per 100ccs.

Subject.	Sample.	Time of Collection.	Result.	Ratio of whole blood to plasma approx.
J. McD.	5ccs. Blood	10.30 am.	14	1/2
J. McD.	5ccs. Plasma	10.30 am.	22	
J. McD.	5ccs. Blood	4 pm.	70	3.5/1
J. McD.	5ccs. Plasma	4 pm.	19	
J. Mc.	5ccs. Blood	10.30 am.	28	2/1
J. Mc.	5ccs. Plasma	10.30 am.	13	
J. Mc.	5ccs. Urine	10.30 am.	22	
J. Mc.	5ccs. Blood.	4 pm.	60	10/1
J. Mc.	5ccs. Plasma.	4 pm.	6	
J. Mc.	5ccs. Urine.	4 pm.	20	



Pt 2830 Pb 2833 Mg 2852

Magnified x 6.

Spectrograms from Cell/Serum Distribution of Lead Investigation. (Case J.McC.)

The increase of the lead content of the cells relative to the plasma content as the day progresses is shown by the spectrogram opposite.

Spectrogram 1, 2 and 3 are those of blood, serum and urine at 10.30am. 4, 5, ^{and} 6, ~~and~~ 7 are those from the same subject at 4pm. Contrasting spectrograms 1 and 2 with 4 and 5 it is evident that the whole blood lead content has increased relative to that of the serum as the day advances. This is best indicated by assessing the density of the platinum line to the lead line; in spectrogram 5 for example the platinum line is much more dense relative to the lead line than in spectrogram 2. The reverse is the case on comparison of spectrograms 4 and 1.

Note also the reduction of iron in the serum and urine spectrograms, compared with those of whole blood, and the heavier concentration of Magnesium in urine as compared with serum. The small dots on all spectrograms are the identification dots mentioned in the text.

(Red Pb. Green Pt.)

The cause of such a variation over such a short period, I consider to be due to the influence of diet and occupation. It obviously provides another source of divergence, in results reported by different investigators i.e., difference in sampling time, and the influence of extrinsic factors of diet, and occupation prior to sampling.

From these results I consider that the cell/plasma distribution of lead is so variable that it is of little diagnostic value, and that those workers who claim that the cells, or plasma, contain all the lead present, are in error in that they fail to take into account the variable distribution which occurs.

From my experimental results I am in agreement that in lead poisoning, the bulk of the lead is to be found in the cells. This is contrary to Aub's findings, but in agreement with those of Kehoe, and Blumberg. There is confirmatory evidence of this from the blood/urine ratios in lead poisoning, where in many cases although the whole blood lead content is grossly elevated, the urine lead concentration remains within normal limits. The variation in the daily urine lead content is compatible with a variable whole blood, or plasma lead content throughout

the day. One point of interest is that while all degrees of relative concentrations may be encountered from 1/6 to 9/1, an instance of gross elevation of both whole blood, and plasma lead contents is lacking.

SECTION VIII.

LEAD CONTENT OF THE TISSUES.

The lead content of the tissues has been the subject of considerable interest for some time. Meilliere following up the conception of normal lead investigated the lead content of human viscera. ⁽¹¹⁴⁾ Barth (1931) investigated the lead content of the bones with findings as to their lead content, confirmatory of those of other workers. The lead content of post-mortem material is not of much value in practice, as the pathological condition from which death occurred doubtless affects the lead content of the tissues, nor will a diagnosis ever be based on ⁽¹¹⁵⁾ post-mortem findings. Kehoe (1933) had the opportunity of studying the lead content of the tissues in a case of instantaneous death following a stab wound to the heart: while it is evident that his method lacks sensitivity due to failure to detect lead in certain organs, his table of results is of interest and is given in full in Table XIV., along with the results in a case of my own, and those of some other workers. It is evident that different workers

evince different interests in the tissues analysed for lead. To me these results are of interest mainly from the lead content of 1) the bones, 2) the lungs, 3) the liver and spleen and 4) the brain.

All workers are agreed that the lead content of the bones is high, and the main site of stored lead in the body. Tompsett pointing out that the short bones had a higher lead content than the long bones considered that their role in the production of spontaneous toxic episodes was more important; their higher lead content he attributed to their greater proportion of red marrow. My experience of analysis of tissues is limited, but I can confirm that the lead content of the short bones is high. Whatever controversy may centre round Aub's theories as to the value of deleading therapy, there is no doubt as to his theories of lead storage, and the existence of a close metabolic relationship in this respect between calcium, lead and phosphorus. In discussing the question of absorption by Inhalation as opposed to absorption by Ingestion (Section II.2.), I drew attention to the low lead content of the lungs as being contrary to the conception that inhalation is the main source of lead absorption. McNally's case died

of lead encephalopathy following the inhalation of lead fumes generated by the burning for fuel of discarded storage battery boxes; yet he gives no figures as to the lead content of the lungs. The results from the hands of other workers are confirmatory of my own findings - the lead content of the lungs in all cases has been found to be low.

(119)

Legge (1912), experimenting on cats showed that lead is absorbed directly into the portal circulation; of all the viscera, weight for weight the liver has the highest lead content, irrespective of cause of death or lead poisoning. The low lead content of the spleen is surprising, since a high lead content could be confidently predicted in an organ where phagocytosis of the erythrocytes was taking place; such lead as is liberated by the spleen must pass into the portal circulation by way of the splenic vein, to be ultimately deposited in the liver. It was considered by earlier workers that Cirrhosis of the Liver was a possible lead lesion, but this was discounted by later workers who attributed it to the alcohol to which lead workers were supposed to be addicted. In view of the agreed findings of all workers on the lead content of this organ it must be admitted that Hepatic Cirrhosis

may have lead absorption as a contributory aetiological factor. If lead causes a sclerosing nephrosis of the kidneys there is no reason to suppose that its action on the liver is different. The lead content of the liver is to me, a further confirmation of free (120) alimentary lead absorption. Kehoe criticising the use of high calcium diets in attempts to enhance lead storage, considers that lead is not bound in a stable form in any but nervous tissues. If this is the case, then the total amount of lead immobilized in the tissues in stable form is low, as indicated by the lead content of the brain. Occasional attempts have been made to assess the lead distribution in experimental animals by use of radioactive isotopes of lead. It is doubtful however if the distribution of a radioactive isotope of lead in the tissues behaves in the same manner as the metal itself. At any rate the use of such isotopes of lead is no longer necessary in view of the sensitivity of modern analytical methods.

TABLE XIV.

Results in Microgrammes of Lead per

<u>Investigator</u>	<u>Liver</u>	<u>Kidney</u>	<u>Spleen</u>	<u>Muscle</u>	<u>Flat Bone</u>	<u>Long Bone</u>
Kehoe	80	70	0	0	1110	-
McNally	5962	62	-	-	-	5570
Tompsett	173	135	168	-	855	-
Weir Lab.	300	5	20	5	500	-
Cholak	-	-	1050	45	16,850	-

(115)
 Kehoe (1933) Fatal stab wound of heart.
 (116)
 McNally (1943) Lead Encephalopathy.
 Weir Medical Dept. (1942)
 Uraemia and Melanosis Colon.
 (117)
 Tompsett (1935)
 Mean results in twenty cases
 with no lead exposure.
 (118)
 Cholak (1943) Not stated.

Note: With the exception of Tompsett none of the other investigators state whether the analysis was carried out on dried tissue, or tissue in the post-mortem state. It has been assumed that these analyses were carried out in the moist condition and I have conformed accordingly. While drying of the tissues allows of more exact sample quantities being analysed, by concentrating the metallic residues in the tissues, higher results are obtained and the influence on the results of the condition causal of death is increased. For the sake of uniformity it would be better if investigators reported results on 100 grammes from the tissues ash - not dried, or moist tissues.

100 grams tissue or 100 cc's., fluid.

Lungs Heart Brain Suprarenals Thyroid Pancreas

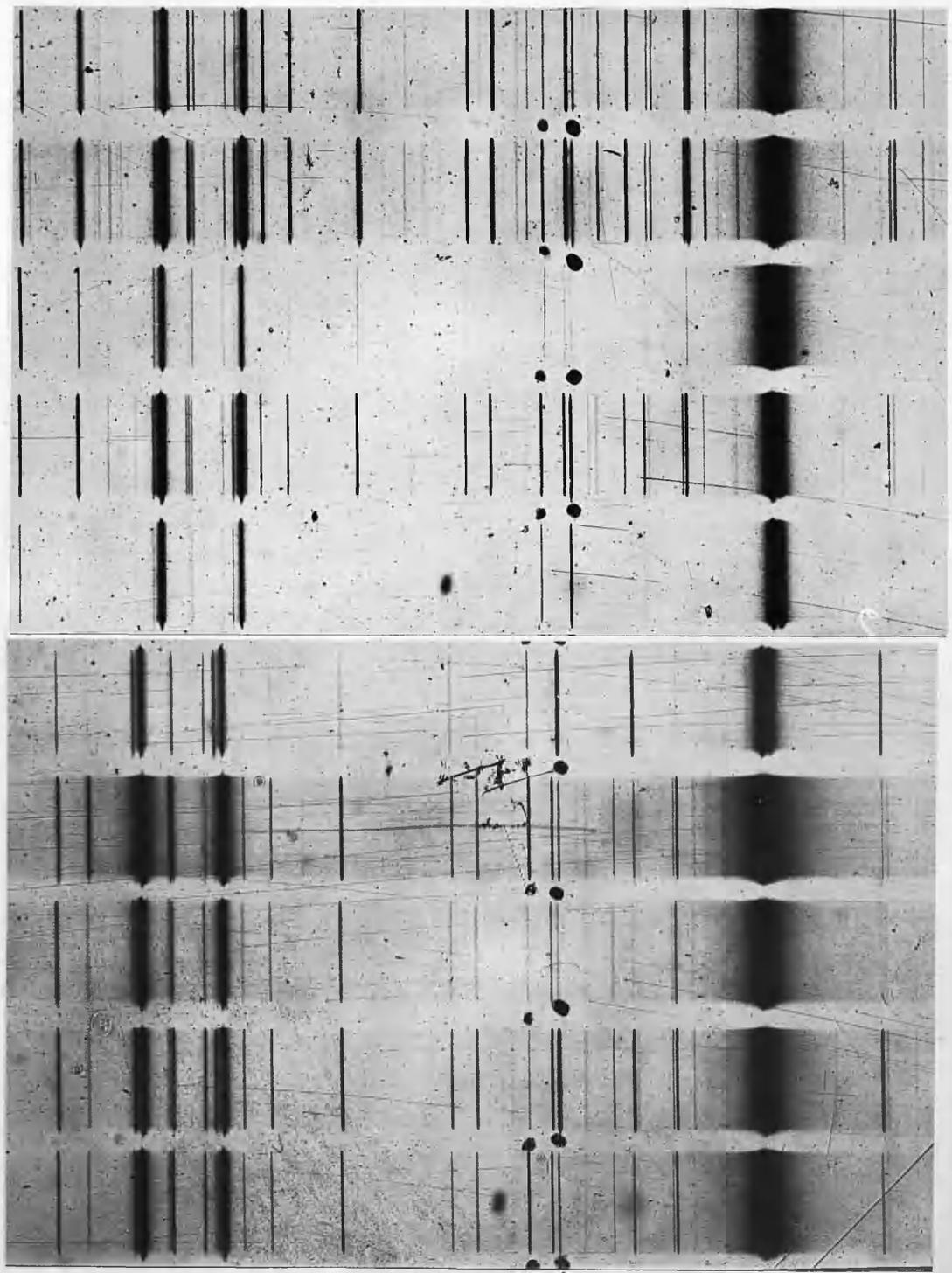
0	0	0	-	-	40
-	-	-	205	-	542
50	-	50	-	-	-
8	16	18	-	-	-
39	50	-	24	62	-

Age 22. Male.

Age 48. Female.

Age 12. Male.

Not stated.



1
2
3
4
5
6
7
8
9
10

Pt 2830 Pb 2833 Mg 2852

Magnified x 6.

- 1. Kidney. 2. Liver. 3. Brain. 4. Muscle. 5. C.S.F.
 - 6. Rib. 7. Lung. 8. Heart. 9. Spleen. 10. Blood.
-

Spectrogram showing the Lead Content of the Tissues from the case cited in the text. It is evident from the density of the Lead Lines compared with those of Platinum that the lead content of the Liver and Bones is high compared with that of Lung or Brain.

- 1. Kidney.
- 2. Liver.
- 3. Brain.
- 4. Muscle. (striped)
- 5. Cerebro-spinal fluid.
- 6. Rib.
- 7. Lung.
- 8. Heart muscle.
- 9. Spleen.
- 10. Blood.

SECTION IX. PART 2.LEAD CONTENT OF CEREBRO-SPINAL FLUID.

Little work appears hitherto to have been done on the lead content of cerebro-spinal fluid. Rabinowitch, Dingwall and McKay (1933)¹²¹ described a qualitative spectrographic method for the detection of lead in cerebro-spinal fluid. Foster, Langstroth, and McCrae (1935)¹²² using a high voltage spark as the method of excitation described a quantitative spectrographic method which they claimed was accurate to $\pm 15\%$. This method presented the novel feature of using the magnesium content of the cerebro-spinal fluid as the internal reference element, since it was always constant for a given quantity of cerebro-spinal fluid. Thus by taking a number of aliquots of cerebro-spinal fluid and adding to them known increasing quantities of lead, by microphotometric measurement of the variation of the line densities of lead to magnesium, it was possible to arrive with fair accuracy at the lead content of the specimen to which no lead had been added. Foster claimed that since no preliminary preparation or treatment of the samples was necessary contamination error was abolished and the results were therefore of the highest accuracy. No figure as to normal cerebro-spinal fluid lead concentrations are given, these workers confining themselves to a description of the method elaborated. By

use of the method described I investigated the lead content of the cerebro-spinal fluid in a series of cases in a large city hospital.

I would draw attention to the following facts when the results are considered. The cases cannot be strictly considered as 'normal' as they had undergone a variable period of hospitalization and treatment, and were all suffering from pulmonary tuberculosis. The samples were collected direct into the crucibles, and covered at once, the blood samples being collected immediately after. The results shown on table XV are those on which I was certain that no contamination had occurred and that exact volume of sample had been achieved; several doubtful samples were discarded.

From the analysis of cerebro-spinal fluid it is evident that lead is normally present in the cerebro-spinal fluid. From the writings of certain workers it would seem that the presence of lead in the cerebro-spinal fluid is regarded as pathological, and its presence considered to be an indication of lead poisoning. This is obviously a serious diagnostic error. The concentration of lead in cerebro-spinal fluid is of interest as it shows a fairly close approximation in concentration to whole blood in several cases, and the ratio concentrations of blood/

C.S.F., and blood/serum are similar. The variations of whole blood lead/cerebro-spinal fluid lead are consistent with the variation in ratio concentrations found between whole blood and other body fluids.

It has not as yet been possible to investigate the lead concentration of cerebro-spinal fluid on lead absorption or poisoning. I consider it probable that the lead content of cerebro-spinal fluid will show a rise in concentration in the same manner as does blood. How much such an increase in the lead content of cerebro-spinal fluid contributes to the manifestations of Acute Lead Encephalopathy is uncertain, although it must play some part. While it is probable that spasm of the small cerebral blood vessels is responsible for many of the convulsive features of such an attack, the lead carried to the nervous tissues by the cerebro-spinal fluid must contribute to it, and initiate it.

Whatever may be the effects of the cerebro-spinal fluid lead on nervous tissues, once it is accepted that lead is normally present in the cerebro-spinal fluid the following important considerations arise.

Where Lead Encephalopathy is diagnosed or suspected, confirmation of the diagnosis should be sought by analysis of the cerebro-spinal fluid. It seems to me sound

therapeutics to treat such an emergency on the same lines as an acute Uraemia, coupled with repeated lumbar puncture, since lead is excreted into the urine and sweat, and is present in the cerebro-spinal fluid.

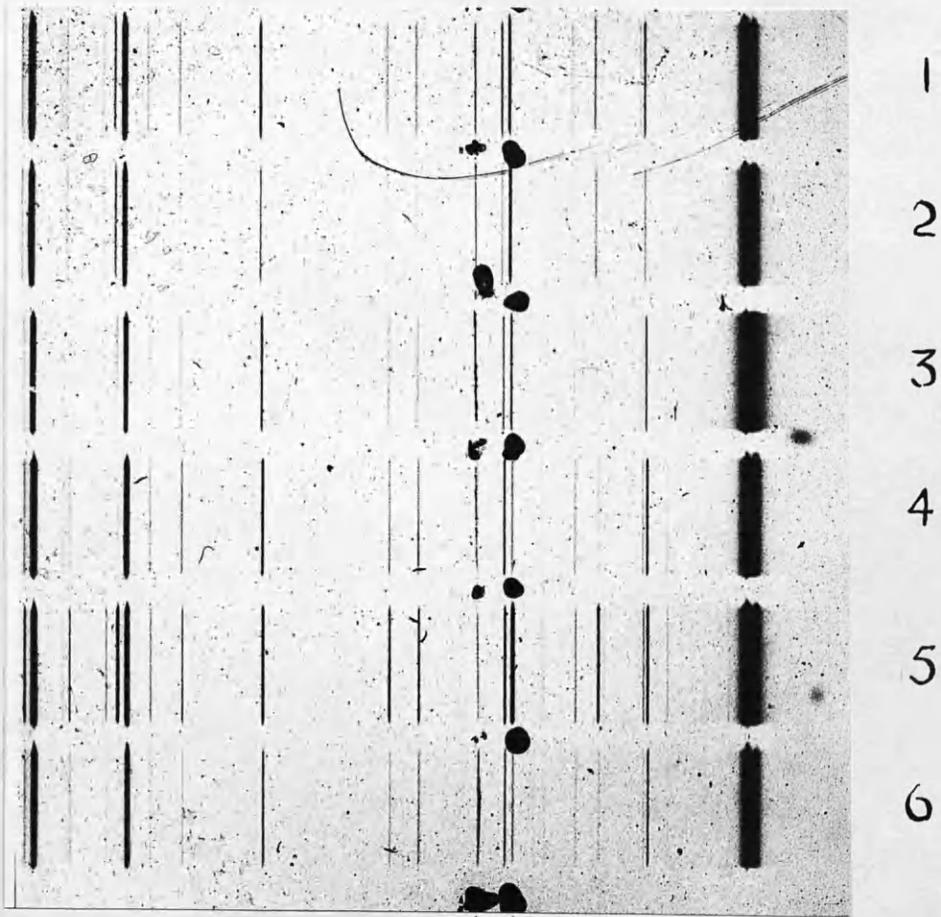
The question of 'Deleading' is often raised from the aspects of Treatment and Diagnosis. It is considered by some workers that a gradual withdrawal of the lead stored in the bones, is preferable to allowing the patient to run the risk of an acute spontaneous mobilization with the associated dangers of acute encephalopathy. In cases of doubtful diagnosis it has been suggested that a 'Test Deleading' will provide proof as to whether the case is one of lead poisoning or not. Normally the response to a specific dose of a deleading agent is slight or absent; in cases of chronic lead poisoning the abnormal degree of lead storage is indicated by a sharp rise in the blood lead concentration. Both procedures are undoubtedly risky and attended by the danger of an acute Lead Encephalopathy often dramatically sudden of onset. In these cases repeated checks on the lead content of the cerebro-spinal fluid would provide an indication as to when the deleading process was initiating dangerous concentrations. From the results shown it would appear that blood analyses alone are not enough in these cases, as the lead content of the

cerebro-spinal fluid may exceed that of whole blood to a considerable degree.

TABLE.XV.

Results in Microgrammes per 100ccs.

<u>Subject.</u>	<u>Blood 5ccs.</u>	<u>C.S.F. 5ccs.</u>	<u>Ratio (approx)</u>
G.B.	120	33	4/1
G.S.	27	40	2/3
M.C.	32	30	1/1
B.B.	5	10	1/2
A.A.	8	22	1/3
D.F.	40	18	2/1
S.T.	60	22	3/1
8.	18	22	1



Pt 2830 Pb 2833 Mg 2852.

Magnified x 6.

Spectrograms of Blood and Cerebro-spinal Fluid.

Each pair (1 and 2, 3 and 4 etc.) are taken from the same subject. Odd numbers are Blood Spectrograms, even numbers are Cerebro-spinal Fluids, which also shows a considerable reduction in its Iron content compared with blood. Note the heavy Magnesium lines in cerebro-spinal fluid indicative of a high content of this metal.

Vir Metallicus, Hypochondrium Dextrum
intentum, Splen Magnus, et, alvus intenta
subdura, Spirituosus, decolor, huic in genu
sinistro recidiva.

Hippocrates. Epidemia.

SECTION X.CLINICAL AND BIOCHEMICAL CRITERIA FOR LEAD POISONING.

So profuse has been the literature on lead poisoning, that the necessity of an accurate analytical method for lead concentrations in blood and tissues might seem superfluous. Perusal of the long list of symptoms and pathological combinations tabulated by Legge (123) (1934) from his unrivalled experience of lead poisoning, shows that this industrial malady may exhibit a clinical picture indistinguishable from that of disease of totally different aetiology. In the event of a history of exposure to lead, it is wrong to assume that lead is inevitably the cause of the individuals disability without recourse to laboratory confirmation, as is not infrequently done, even by physicians of repute. Further stress on the necessity of laboratory confirmation is evidenced by the fact that considerable disagreement exists between experts on the value of the clinical symptoms and signs which have hitherto been regarded as pathognomonic of lead poisoning.

(124)

Reznikoff (1940) considers that the following are the cardinal symptoms and signs of lead poisoning.

- (I) Anaemia.
- (II) Typical Colic.
- (III) Paralysis.
- (IV) An excess of one hundred microgrammes of lead per litre of urine.

LEAD ANAEMIA.

According to Aub's theory, lead once absorbed becomes attached to the red cell envelope, with a resulting marked increased red cell fragility. The Erythrocytes accordingly break down much more readily, and anaemia results. ⁽¹²⁵⁾ Legge (1912), and ⁽¹²⁶⁾ Reznikoff consider that the anaemia is of a severe haemolytic type; jaundice however is not a typical finding in lead poisoning, and an indirect positive Van den Bergh reaction appears to be seldom found. In accepting Aub's theory, ⁽¹²⁷⁾ Collier draws attention to the fact that the blood picture may vary dependent on:-

- (I) The severity of the exposure.
- (II) The duration of the exposure.

An overwhelming exposure to lead, may produce an anaemia before the bone marrow can respond by producing reticulocytes, so that a severe anaemia without punctate basophilia may be found; on the other hand a chronic exposure may have rendered the marrow no

longer capable of response, and a blood picture resembling that of Aplastic Anaemia results.

Punctate Basophilia is still considered to be one of the most typical, and constant features of the blood picture in lead poisoning, but it is not invariably encountered, and may occur in other diseases.

(128)

Lane (1936) considers that up to two thousand punctate basophils per million erythrocytes may be encountered in lead workers who do not show symptoms. He further considers that the size of the basophilic granules is of importance - the larger the granules the more serious the prognosis. Sanders (1943) considers the maximum normal number of basophilic cells to be rather higher than Lane - five to nine thousand per million erythrocytes, and is of the opinion that the size of the granule is of no diagnostic significance being dependent on the number of basophilic cells present, and unrelated to the stimulus producing them.

(129)

The Burtonian or lead line on the gums is not specific to lead, occurring also in bismuth, and mercury poisoning; I have frequently observed this blue line on the gums of Indian seamen, where it was attributed to copper derived from cooking utensils. Reznikoff

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considers that the lead line is more discrete, and less granular than the bismuth line and that it is pathognomonic of lead absorption. Johnstone on the other hand, states that it is neither a line, nor is it blue, but a series of gray black specks, and of no significance in indicating how much lead is present in the body. Legge finally states that this lead line, without in any way altering the conditions pertaining to lead exposure, could be diminished or entirely abolished by the exercise of dental care.

Where paralysis is present, diagnosis of its origin may be difficult. Reznikoff however considers that true paralysis (presumably paralysis confirmed to be caused by lead), is rare. In sharp contrast to this, (130) Legge (1934) considers that the best routine spot test among lead workers, is to test the power of grasp of the fingers, and pressure against resistance of the wrists. I cannot agree that this is a sound practice. I have noticed, particularly in Buffers and Dressers, a weakness in the wrists and fingers which has nothing to do with the small amounts of lead absorbed in the course of their daily work. It is a Chronic Pressure Atrophy arising from the prolonged use of high speed wheels and drills.

Manifest lead poisoning, such as is encountered in its gravest form Lead Encephalopathy would not at first sight require laboratory confirmation especially in an industrial worker. The symptoms however are difficult to differentiate from Influenza or Tuberculous Meningitis, and encephalopathy may arise from other metals e.g., Arsenic.

Typical Lead Colic is considered to affect mainly the upper half of the abdomen, and to occur in short sharp attacks, lasting about half an hour (Legge). From experimental ingestion of lead, I can confirm this in full agreement with John Hunter's description of "dry belly ache". The discomfort was experienced mainly above the umbilicus, and in the region of the caecum.

This divergence of expert opinion on the value and constancy of certain cardinal clinical criteria, probably originates in factors similar to those influencing variation in the biochemical findings. The duration and severity of the exposure, the age of the patient, and his state of health, the type of work on which he has been engaged, may all give rise to such an atypical clinical picture, that recourse must be taken (however unwillingly) to reliance on the laboratory findings.

The range of normal blood concentrations has been discussed, and for the sake of comparison, my findings are tabulated with those of other investigators in Table XVII. From this comparison, while the lower limits are variable to a considerable degree, the more important feature of the normal maximum blood lead concentration is in fair agreement. This normal maximum is one hundred microgrammes per hundred cubic centimetres of blood. I am of the opinion along with Blumberg, that the critical concentration above which symptoms may be expected to appear, lies between one hundred and one hundred and fifty microgrammes, increasing in severity as the concentration rises above this latter figure. This applies to chronic lead poisoning only, as I have shown the blood level may be much higher without symptoms in acute cases.

It is essential that a diagnosis should not be based on analysis of a single sample, and I would recommend where possible that the results be obtained on three samples at intervals of two days. Apart from an acute lead encephalopathy, this delay need make no difference to the treatment. Blumberg (1935) has shown

TABLE XVII.

Results in Microgrammes per 100ccs. blood.

Investigator	Date	Method	Results	Critical Concentration
Kehoe Thamman and Cholak.	1935	S.Di-Phenyl -Carbazide	60-100	-
Tompsett and Anderson	1935	Dithizone Method	50-70	-
Willoughby and Wilkins.	1938	Dithizone Method	0-90	-
Cholak and Bambach.	1943	Dithizone Method	25-90	-
Blumberg and Scott.	1935	Spectrographic Method	25-100	100-130
Kehoe Thamman and Cholak.	1935	Spectrographic Method	30-70	-
Cholak and Bambach.	1943	Spectrographic Method	25-100	-
Tracy and McPheat.	1943	Spectrographic Method	11-100	100-130

that in lead poisoning, lead is slowly excreted; there is no fear therefore, that the delay caused by repeated analysis will lead to errors in diagnosis due to low results obtained in the latter analysis arising from excretion. In health as I have shown, (Table IV) lead is eliminated almost as rapidly as it is absorbed.

The normal ratio of blood lead to urine lead is not so well agreed upon, but the divergence may lie to some extent, in the differences of sampling methods employed. The maximum normal concentration laid down by Kehoe of one hundred microgrammes per litre will, in my opinion, be often exceeded in health. The normal ratio of 10/1 for blood lead to urine is, to me, representative of too great a difference in the lead concentration of blood and urine. In my experience the normal ratio is from 2/1 to 6/1 with a tendency to the lower ratio irrespective of occupation. This is confirmed from the results of other workers, e.g., Tompsett. What seems clear, is that in lead poisoning, the blood lead concentration is much higher relative to the urine lead concentration than is met with in health. A high blood lead and a disproportionately low urine lead is a better indication as to lead poisoning than any arbitrary

figure based on excretion alone, especially when the rate of excretion is correlated with the blood lead concentration as indicated previously. In healthy individuals who do not complain of symptoms, a high blood lead is always accompanied by a high urine lead. The distribution of lead between the cells and the serum, does not give data sufficiently constant for any diagnostic significance to be attached to them. While the distribution of lead between the cells, and the serum is variable, there seems little doubt that on abnormal lead absorption, the bulk of the lead is rapidly adsorbed to the cells. It is clear also that lead is present in the serum under normal conditions, and to regard this fact as an abnormality is entirely in error. Johnstone in stating that no lead is normally present in ten grammes of serum, is in effect saying that no lead is normally present in the serum at all. In accordance with Aub's theory as to the action of lead in producing a severe haemolytic anaemia, in lead poisoning, I consider it probable that an increase of the serum iron and urine iron will be met with. Spectrographically this would be at once detectable owing to the low amounts occurring normally. German and French workers stress the presence of Haematoporphyrinuria in lead poisoning. As

yet I have not advanced the investigation sufficiently to give results, but I consider that the estimation of Haematoporphyrin by absorption spectrography to be practical. Acid Haematoporphyrin has definite absorption bands in the red-orange spectrum, about 6000 A^o, and a broad band in the yellow-green 5300 A^o. The quantitative estimation depends on the degree of absorption by Haematoporphyrin in varying concentration of the bands of light emitted by the electrodes, when the Haematoporphyrin solution is placed between the electrodes and the spectrograph slit. Once completed the method should confer advantages over that described (132) by Heilmeyer (1943) using the Zeiss Pulfrich photometer, where the preparation of standards is laborious and the sensitivity of the instrument limited.

The question is often raised in doubtful cases of lead poisoning of a "test deleading" by the use of parathormone injections, or ammonium chloride orally. The rationale underlying this procedure is that in chronic exposure to lead, storage of lead occurs to an abnormal degree in the long bones. This abnormal storage of lead is manifested by an excessive rise in the blood lead concentration in response to a definite dose of the deleading agent. Normally the response is slight or

absent, but in chronic lead poisoning a sharp rise in the blood lead concentration occurs. This procedure is not regarded with favour by most experts, and can only be attempted where the blood lead concentration is not unduly elevated, and the haemoglobin and red cell counts are about 80% of normal. The procedure is undoubtedly risky, accompanied by the danger of an acute toxic episode, and must inevitably aggravate the patients illness for at least a short period. Where deleading is carried out for diagnostic or therapeutic purposes, I consider that repeated checks on the cerebro-spinal fluid concentration should be carried out. Since lead is excreted in the sweat, it seems to me that treatment on the same lines as an acute Uraemia would more adequately control the effects of the lead mobilized from the bones, since its elimination would be considerably accelerated. Where Lead Encephalopathy is diagnosed, confirmation should be obtained by analysis of the cerebro-spinal fluid. If this is done then errors such as I have encountered should not be made, where a diagnosis of Lead Encephalopathy was pronounced and the cerebro-spinal fluid was subsequently found to contain twenty two microgrammes of lead per hundred cubic centimetres.

Where it is not possible or convenient to obtain samples of all biological materials, on which material is it preferable to carry out analysis. ⁽¹³³⁾ Kehoe (1933) preferred to carry out analysis on a litre of urine from the total daily urinary out-put, considering that the result was indicative of both the mean blood and urine concentrations. He arrived at his conclusions on the assumption that the ratio of blood lead to urine lead was ten to one. As I have indicated I do not agree with this ratio, and the procedure fails to recognize that the blood and urine concentrations do not run parallel. ⁽¹³⁴⁾ Litzner and Schmitt (1933) prefer to analyse blood samples ⁽¹³⁵⁾ as do also Tompsett and Anderson (1939), and the majority of other workers.

The choice of analytical material is largely dictated by the sensitivity of the analytical method employed. I always choose samples of blood correlating the result with urine as described. By reason of the small amounts of analytical material required, no difficulty has been as yet encountered in obtaining analytical material when the spectrographic method was employed.

Among the numerous symptoms and signs encountered in lead poisoning the following are what I consider to be the cardinal symptoms and signs of lead poisoning.

"A Metallic Man has his right hypochondrium bent, a large spleen and a costive belly, he breathes with difficulty, is of a pale and wan complexion and is apt to have swellings in the left knee."

Hippocrates.

Epidemia.

1. Anaemia; no case of lead poisoning has ever been encountered without anaemia of varying severity. The blood picture will not always conform to the classical description of secondary Microcytic Anaemia with Punctate Basophilia in marked evidence.
2. Lead Colic; since a considerable quantity of absorbed lead has been previously ingested, lead Colic is rarely absent in lead poisoning.
3. A blood lead concentration in excess of one hundred and fifty microgrammes per hundred cubic centimetres of blood is always abnormal when confirmed by repeated analysis. Between one hundred and one hundred and fifty microgrammes per hundred cubic centimetres of blood sub clinical lead poisoning is present, and indicates that a dangerous concentration is being reached.
4. A high blood lead and a disproportionately low urine lead is strongly suggestive of lead poisoning.
5. A high blood lead with the bulk of the lead in the cells is suggestive of lead poisoning, and may be taken as confirmatory of the other findings.
6. A high blood lead with a correspondingly high lead content of the cerebro-spinal fluid is strongly suspicious of lead poisoning, the latter may be

taken as indicative of the amount of lead available for attack on nervous tissues in cases of lead Encephalopathy and Paralysis.

To these cardinal symptoms may be added the numerous other manifestations of lead poisoning, simulating the symptoms and signs of other diseases, and depending for their manifestation, on the type of exposure, the individuals age and habits, and occupational pursuits.

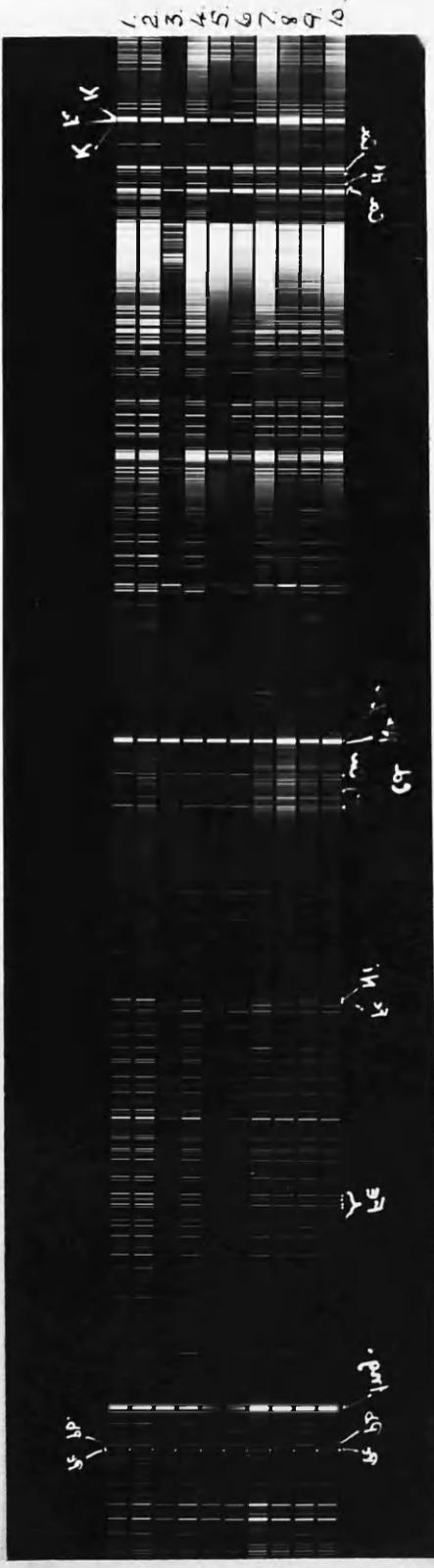


Plate of unmagnified spectrograms as seen on developed plate.

The reproduction of spectrograms is difficult and comparatively seldom attempted; in many cases presentation is given as is seen in plate 1 where the negative is used. e.g. Sheldon and Ramage (Biochemical Journal 1931, 25, 1608) The spectrum lines of the metals on the negative therefore appear as bright lines on a dark background. Plate 2 shows the appearance as seen on the developed plate which is viewed at magnification on the Judd Lewis Comparator.

These spectrograms are those of the tissues analysed from the case quoted in the text Section VIII Part 2. The metals present are marked on the spectrogram; it is seen that many of the lines are due to the presence of Iron. Note the Cadmium line which is present in Kidney and Liver, and absent in the other tissues, also the mutual overshadowing of Sodium and Zinc (3300 \AA°) mentioned in the text. (Page 68)

These plates also show the manner by which identification is achieved by the method of alignment with standard plates. It is evident that alignment of the prominent lines Magnesium (2852 \AA°), Copper (3247 \AA° and 3274 \AA°), Iron (3100 \AA°) enables rapid identification of all the other element lines possible. Note also the dense Cyanogen band which arises with use of graphite electrodes.

- | | | | | |
|------------|-----------|-----------|------------|------------|
| 1. Kidney. | 2. Liver. | 3. Brain. | 4. Muscle. | 5. C.S.F. |
| 6. Rib. | 7. Lung. | 8. Heart. | 9. Spleen. | 10. Blood. |

TABLE XVIII.

<u>CONSTANT</u>	<u>VARIABLE</u>	<u>PREDOMINANT</u>	<u>ABSENT</u>
Iron	Silver	Iron	Arsenic
Calcium	Nickel	Calcium	Gold
Potassium	Chromium	Potassium	Platinum
Sodium	Antimony	Sodium	Mercury
Magnesium	Bismuth	Magnesium	Thorium
Zinc	Manganese	Zinc	Barium
Silicon	Vanadium	Copper	Uranium
Copper			Cobalt
Tin			Molybdenum
Aluminium			Tungsten
			Thallium
			Titanium

SECTION XI.

METALS ASSOCIATED WITH LEAD IN BIOLOGICAL MATERIAL.

Spectrograms of blood and other biological material using the wave band between 2700 A° - 4700 A° reveals the presence of a large number of metallic elements. Some of these elements are unfailingly present, others variable in their appearance. To those elements which are constantly present I have given the appellation physiological metals assuming that their constant appearance denotes function, to the others the title "Occupational Foreigners" on the assumption that this is their origin. All the metals which are present in blood are present in all other biological material although in the latter considerable reduction in the amount present may be found. Table XVIII, shows the metals which I have encountered in blood and biological material and the group to which I consider they belong. It should be remembered that volatile metals like mercury and sulphur would be driven off during the ashing process. The presence of silicon is doubtful probably originating in the crucibles used for ashing. All the metals present in whole blood are present in all other biological fluids and tissues. Iron is strikingly reduced in all the other fluids apart from blood, and a relatively small quantity of all the other metals appears

to be carried in the cells with the exception of lead. Copper and Magnesium for example are much stronger in serum and urine than are iron and lead. As far as can be ascertained from a visual comparison of line densities, there is no particular metal which one could say with certainty was the dominant in the other biological fluids. Urine perhaps shows rather heavier lines of sodium and potassium than does serum; the density of the magnesium spectrum in cerebro-spinal fluid is rather indicative of a relatively higher concentration of this metal than is met with in the majority of the other biological fluids (blood excepted).

The conception of the role played by the trace elements in health has been considerably stimulated by the experiences of veterinary science. "Sway back" and "Pining Disease" in sheep is now recognized to be a disease due to copper deficiency. Molybdenosis in cattle presents the other aspect of an excessive concentration of a trace element setting up disease.

In the human subject the trace elements have had attributed to them the role of oxygen carriers (Iron) and Acid/Base, Equilibrium Regulators (Zinc), and a large speculative group, catalysts for certain biochemical

reactions. Function is considered to be achieved by the stoichiometric combination of the metal and cell proteins to form a specific metallo-protein; excess or deficiency of the trace elements may therefore give rise to metabolic upset.

Nothing is known as to the function of lead, but a considerable number of reports are available as to the effects of lead on the experimental animal. These reports have been mainly directed to a study of the noxious effects of the metal. It is credited with markedly reducing the hydrogen transfer and glycolysis in brain tissue. In rabbits the formation of haemolysins and agglutinins is greatly increased, but the formation of precipitins is retarded. In rabbits also the formation of diphtheria antitoxin is said to be accelerated on an increased lead intake.

In Man and rabbits the effect of an increased lead intake on the red blood cells is indicated by the production of punctate basophils; this phenomenon is not demonstrable in the blood of cats and goats. This appearance of stippled cells has been considered to be an indication of susceptibility to lead in the species exhibiting it (vide Lancet, 1939). I consider that the reverse is probably more correct. Legge used cats as his experimental

animals since he had observed that it was impossible to keep a cat in a room where a lead process was being carried on - they developed lead poisoning and rapidly died.

The production of punctate basophils and nucleated red cells should be taken as indicative of the response of the bone marrow to the anaemia produced by lead absorption, rather than as a response to the lead itself. Why otherwise does severe lead poisoning frequently occur without punctate basophilia being a prominent feature of the blood picture.

In some respects there is biochemically a close similarity between iron and lead. Both are stored mainly in the bones and the liver, both metals are principally concentrated in the cells rather than the serum. (137) McCarthy (1943) commenting on the normal diurnal variation in the haemoglobin concentration in blood, draws attention to the progressive diminution in this concentration as the day advances. Some workers have reported this variation to be as high as 20%. As I have indicated (Section VII.2.) the reverse behaviour was found in the case of lead, where the whole blood lead, and cellular lead content increased in the course of the day. It is possible that these two findings are in some way connected.

Clinically the results of experimental observations on the effects of lead on unstriped muscle are of interest.

The typical lead colic is no doubt what is reproduced experimentally when the contractility of unstriated muscle is increased on exposure to lead in vitro. A similar result has been observed on the muscle in the wall of the blood vessels. To this may be attributed two facts of centuries old recognition in connection with lead poisoning - the tendency in lead workers to Hypertension and Cerebral Haemorrhage, and the "leaden" pallor of the face. While anaemia may contribute to this pallor, the typical saturnine facies is considered to be mainly due to contraction of the superficial blood vessels. One cannot help wondering if the addiction to alcohol which Legge attributes to lead workers was not their way of unconsciously attempting to combat the subjective manifestations of the metal which was slowly poisoning them.

It cannot be ignored that lead is constantly present in the tissues and body fluids in health, and that there is a physiological mechanism for its storage. It is also literally unnatural that a "toxic foreigner" should be transmitted from mother to foetus in the same concentration, and that the infant should retain it, if it has not a definite function. The knowledge of trace element function is as yet imperfect, but if lead has a definite function

it occupies a unique place among the trace elements. It is the only functional trace element within our knowledge which in excess concentration is capable of producing a clinical syndrome meriting the title of a Disease....."Now the Potters make use of burnt and calcined Lead for glazing Ware; and for that end grind their Lead in Marble Vesiels by turning about a long Piece of Wood hung from the Roof with a Square Stone fasten'd to it at the other end. While they do this as well as when with a pair of Tongs they daub their Vesiels over with melted Lead before they put them into the Furnace; they receive by the Mouth and Nostrils and all the Pores of the Body all the virulent parts of the Lead thus melted in Water and disolv'd, and thereupon they are seized with heavy Disorders; for first of all their hands begin to shake and tremble and they soon become Paralytick, Lethargick, Splenetick, Cachetick, and Toothless, and in fine you'll scarce see a Potter that has not a Leaden Deathlike Complexion.

Now such are the Diseases that torture those imploy'd in Potters Work-houses to manage the Lead. And indeed it is a wonderful thing that Lead which affords such a stock of wholesome Remedies both for internal and external Uses should harbour in its Bosom such Wicked Seeds."

Ramazzini, De Morbis Artificum Diatriba.

Chapter 5. "De Morbis Figulorum." (1703)

SUMMARY.

SECTION I. PART I.

1. The methods in use for the analysis of Lead in blood and biological material are reviewed. It has been found that Electrolytic methods of analysis are inaccurate by reason of incomplete electrolytic deposition of lead. The modern advance on electrolytic methods by use of the dropping Mercury Cathode is more accurate.
2. Chemical methods as elaborated by Fairhall, and Tompsett and Anderson, are criticised mainly on the grounds of inaccuracy due to contamination error by reason of the numerous chemical reagents involved, the prolonged operational time, and the necessity of constant supervision.
3. Lack of sensitivity of these methods makes repeated analysis impracticable by reason of the large amounts of analytical material required.

SECTION II. PART I.

1. The history and Evolution of the modern quartz spectrograph is reviewed.
2. Special attention is directed in this connection to the work of Newton (1672), Fraunhofer (1817), Kirchoff and Bunsen (1860), Angstrom (1869), and De Gramont (1907)

It is seen that the modern spectrograph has advantages in its use ensuring accuracy of working.

3. The operation of the spectrograph is described showing the manner in which the optical elements embodied in the spectrograph achieve the purpose of recording the emission spectra in a dispersed measurable state on the photographic plate.
4. The use of the Judd Lewis Comparator and the identification of the lines of the elements by the method of "Alignment with a Standard Plate", is described.

SECTION III. PART I.

1. The Qualitative analysis of Blood for Lead by spectrographic methods is described; particular attention is directed to the method of collection of samples, and the preparation of the analytical material for spectrographing.
2. The standard spectrographic conditions used in the earlier investigations are described.
3. It has been found that for various reasons the Lead Line 2833 \AA is the most suitable line to use for the analysis of lead in biological material. The principal reasons determining the use of this line are that it is not overshadowed, that it is an 'r,u'

line of Lead, and its proximity to the heavy Magnesium line at 2852 A⁰ renders its recognition easy.

SECTION IV.

1. The methods of Quantitative Analysis for Lead in biological material are reviewed and criticised.
2. The theory of the "Method of Estimation by Use of an Internal Reference Element" is described.
3. The Quantitative Analysis of Lead in Blood using Bismuth as the Internal Reference Element, and a synthetic lead free Blood Ash from which to prepare standards for preparation of the graphs is described.
4. The theory and use of the Microphotometer in its application to the measurement of line densities is also described.
5. The preparation of the graph and result of checks on its accuracy are described showing that the error was usually around ± 3 microgrammes per 100 ccs., of blood.
6. The reasons why I discarded Bismuth as the Internal Reference Element are given, and the dangers of inaccurate results pertaining to its use discussed. The absorption into the circulation of ingested Bismuth is proved, and the distance between its line at 2898 A⁰, and that of Lead at 2833 A⁰ is pointed out as having a further effect in producing inaccurate results.

7. The characteristics of the ideal Internal Reference Element are given and the elaboration of the use of Platinum as the Internal Reference Element is described.
8. Herein also is described the checks for accuracy of the graph prepared, using Platinum as the Internal Reference Element.
9. Attention is drawn to the possibilities pertaining to the use of Platinum in the spectrographic analysis of a number of other metals implicated in Industrial Metallic Poisoning.
10. The variation in results when three operators spectrograph and measure a series of identical samples is shown to be not more than ± 8 microgrammes per 100ccs., of blood. The accuracy of the method elaborated for analysis of lead in blood is considered to have been proven from the results obtained on repeated checks on the graph (Lead curve 4)
11. The application of lead curve 4 to the quantitative analysis of Serum, Urine and Cerebro-spinal Fluid is described. The technique employed in the overcoming of the danger of inaccurate results due to the weak base spectrum of these fluids is given.
12. The result of checks of the graph on Serum and Urine using the method of synthetic ash additions shows that the maximum error is ± 8 microgrammes per 100ccs. The reasons

for lack of data on checks for the accuracy of "Lead Curve 4" in its application to Cerebro-spinal Fluid are given, and attention is drawn to the fact that as yet I have no definite proof as to the accuracy of the results obtained, that they are accurate is considered to be a reasonable assumption.

- 13. The application of the method to Viscera, Hair and Finger Nails is also given.

SECTION V. PART I.

- 1. The sources of Error pertaining to the use of the method are described, and the steps taken to overcome each source of error.
- 2. It is seen that the main Sources of Error are those due to Contamination, Loss of Analytical Material, and inexact Sample Volume.
- 3. It is claimed that by use of the method described the danger of inaccurate results from these sources is reduced to a minimum.

SECTION VI. PART I.

- 1. Herein are described some Spectrographic Facts of importance in the application of the Spectrograph to the Analysis of the "Trace Elements" in biological material.
- 2. The desirability of working between 2700 A°- 4300 A° is discussed.

3. Attention is directed to the advantages pertaining to the use of Graphite Electrodes, and the possible difficulty liable to be encountered due to the Spectrographic phenomenon of Overshadowing.
4. The effect of Variable concentrations of an element in the production of 'a' and 'b' lines is described with the relevant application to trace element analysis in biological material.
5. The conditions which I consider to govern the use of a metal as the Internal Reference Element are given.
6. The dangers pertaining to the use of "Edge Lines" and the phenomenon of Plate Eccentricity are described.
7. There follows a short discussion on the necessity for Strict Adherence to Standard Spectrographic Conditions, and the wide field of Application of Spectrographic Methods of Analysis as an adjuvant to Diagnosis in certain Diseases and Clinical problems.
8. It is claimed that Spectrographic Methods of Analysis are superior to Chemical methods in the analysis of Lead in biological materials, and that the spectrographic method described is superior to previous spectrographic methods elaborated.
9. The advantages over Chemical Methods lie in the Marked Reduction of Operational Time, the elimination of

Contamination Error, the high Analytical Sensitivity of the method and the small amounts of Analytical Material required.

10. The advance over previous spectrographic methods lies in the use of a Microphotometer to measure the line densities, and the use of Platinum as the Internal Reference Element ensuring accuracy of results.

SECTION I. PART 2.

1. The divergence of results reported at the hands of competent observers is commented on.
2. The Variation in Normal Blood Lead Concentrations, and the evidence in favour of the theory that there is a Hereditary Susceptibility to Lead Poisoning is given.
3. The Blood Lead of newly born infants, that of their mothers, and the Lead Concentration of human milk and infant urine is described. From this investigation the following conclusions are drawn.
 - I. The blood lead at birth approximates closely to the maternal blood lead concentration.
 - II. There is a free transmission of lead, (and the other metals) in the maternal circulation through the placenta to the foetus.
 - III. Care in Antenatal Administration of these metallic elements therapeutically would seem to be indicated.

- IV. Lead is secreted normally in human milk.
- V. There is no evidence that the infant excretes the lead inherited, and it is apparently retained.
- VI. The evidence **supports** the theory of a Hereditary Susceptibility to Lead Poisoning.

SECTION II. PART 2.

- 1. The sources of Lead Absorption are discussed.
- 2. The dictum that Inhalation is the main source of lead absorption is criticised, and the evidence against it discussed. It is pointed out that Lead Dust inhaled will to a considerable extent be subsequently coughed up and swallowed.
- 3. The Absorption of Lead from the Intestine is discussed, with comments on the theory that little or no lead is absorbed from the intestine. The difference between Chemical Solubility and Physiological Solubility is pointed out.
- 4. An experiment involving the ingestion of lead is described, from which it is concluded.
 - I. That there is free absorption of lead from the Alimentary Tract.
 - II. That it is possible to increase the lead concentration of blood as much as twenty times without symptoms arising.
 - III. A high degree of tolerance exists as to the effects of absorbed lead which I attribute to the

"Hereditary Factor" described rather than Acquired Immunity.

- IV. The free Absorption of Lead from the Intestine requires that industrially strict attention to Statutory Rules and Orders for Lead Processes should be observed.
- V. The Absorption of Lead through the skin is not considered to be of importance with a possible exception in the case of those who work with fat soluble compounds of lead e.g., Tetra-Ethyl Lead Workers.

SECTION III. PART 2.

1. The Channels whereby Lead is eliminated from the body are discussed.
2. Analysis of the Lead excreted by the Intestine is not considered to be of diagnostic value since a considerable amount of the lead content of the faeces is derived from unabsorbed lead.
3. The normal concentration of Lead in Urine is considered to range from 30 microgrammes to 300 microgrammes per litre of urine. It is recommended that the results obtained from analysis of urine be correlated with the result of a blood analysis obtained about the same time as the sample of urine.

4. Attention is drawn to the fluctation of the Lead Concentration in Urine throughout the day, and the danger of inaccurate analytical data being obtained on analysis of single samples of urine.
5. Experiments are described in the investigation of the Excretion of Lead in the Sweat; from these experiments the following conclusions are drawn.
- I. Lead is excreted normally in the Sweat in small amounts.
 - II. When the blood lead concentration is elevated an increased elimination of lead takes place in the sweat.
 - III. It is suggested that this may be of value in the treatment of Lead Poisoning where on Diaphoresis being induced more rapid elimination of the lead in the circulation would be achieved.
 - IV. Since the excretion of lead in the sweat cannot be accurately estimated, Balance Studies with the object of assessing Lead Storage are of doubtful value.
6. The excretion of Lead in Bile is demonstrated; it is considered that the lead content of Liver Bile is probably rather less than that of urine.
7. The excretion of Lead in the Hair and Finger Nails is also described.

8. The Analysis of foodstuffs indicates that their lead content renders available for absorption from the intestine an amount of lead considerably in excess of .5 milligrams daily.

SECTION IV. PART 2.

1. The range of Normal Blood Lead concentrations reported by different observers is discussed.

2. From a series of eighty cases I conclude that the Normal Blood Lead Concentration is from 11 microgrammes to 100 microgrammes per 100ccs., blood with a critical concentration of 130 microgrammes per 100 ccs., blood. The majority of the results range from 30 microgrammes to 50 microgrammes per 100 ccs., blood, with a mean of 40 microgrammes per 100 ccs., blood.

3. The influence of Extrinsic factors such as the sensitivity of the Analytical Method employed, and Contamination Error in producing such diverse results from different workers is commented upon.

SECTION V.

1. The influence of Occupation in causing elevated Blood Lead Concentrations is shown.

2. It is concluded that Metal Workers and those exposed to the inhalation of Metallic Dusts have a higher blood lead than Office Workers and the like.

- 3. The influence of Occupation in causing a temporary elevation of the blood lead is commented on from the aspect of Errors in Diagnosis.
- 4. Other factors beyond analytical control in causing fluctuation in the blood lead level are the Lead Content of the Diet, and the Lead excreted in the Sweat, and it is considered that the effects of these two latter factors is of more importance than is generally recognized.
- 5. The effect of these various factors in causing fluctuation in the Blood Lead Concentration of the Individual is shown by the results of repeated Analysis on the same three subjects over a period of seven weeks; the variation within normal limits may be as great as $\pm 100\%$.
- 6. The Danger of Diagnostic Error where reliance is placed on the analysis of a Single Blood Sample, and the desirability of Hospitalization and Investigation under Standard Conditions is illustrated by the case history of one of my own cases.
- 7. It is further concluded that the indication of Normal Blood Lead is the presence of lead in the blood in a concentration not exceeding 100 microgrammes per 100 ccs., blood; this follows since the blood lead concentration varies so widely within normal limits in health, in response to the influence of the factors mentioned.

SECTION VI. PART 2.

1. From a large series of cases it is concluded that the ratio of normal Blood Lead to Urine Lead is 6/1 to 2/1 with a tendency to the lower ratio irrespective of occupation.
2. This is in disagreement with the ratio stated as normal by Kehoe, but it is concluded that comparison is not applicable by reason of the doubtful validity of Kehoe's results due to errors in Time of Collection of Analytical Material.
3. I conclude that the Blood Lead Concentration and Urine Lead Concentration do not run parallel, but also that increased lead concentration is balanced by more vigorous excretion by the Kidney.
4. From the experiment on Lead Ingestion I conclude that increased absorption of lead gradually outstrips the power of the kidney to cope with the increased lead concentration so that a high blood lead and disproportionately low urine lead results. This is confirmatory of what has been found in Lead Poisoning - the greater the disproportion between blood and urine lead concentration, the more severe the toxic effects.
5. To obtain accurate comparison results it is recommended that the Bladder be emptied half an hour before blood/

collection, and the sample of urine obtained from that passed about half an hour after blood collection.

SECTION VII. PART 2.

1. The distribution of lead between the Cells and Plasma reported by various investigations is reviewed. It is seen that considerable diversity of results has been reported. The possible bearing of different methods of sampling on reported results is commented upon.
2. From a series of 7 cases I conclude that there is considerable variation in Cell/Plasma Partition of Lead, the results ranging from 9/1 to 1/6.
3. It is therefore concluded at variance with other workers that there may be more than 15% of the whole blood lead content in the plasma.
4. I agree that in Lead Poisoning the bulk of the lead present in whole blood is to be found in the cells, proving this from the results of experimental investigation.
5. The possible effects on results of a break-down in the Erythrocytes with subsequent liberation of lead into the plasma are pointed out.
6. It is shown that in the course of the day there is a change in the Cell/Plasma Distribution of Lead; as the day progresses the lead in the circulation tends

to become fixed to the cells.

7. It is concluded that this variation is due mainly to the influence of Diet and Occupation.
8. It is concluded that the Cell/Plasma Lead Distribution is so variable as to be of little diagnostic value except in so far as the bulk of the lead present in Lead Poisoning will be found to be fixed to the cells.

SECTION VIII. PART 2.

1. The distribution of Lead in the Tissues as reported by different investigators and in a case of my own is shown.
2. Analysis of the reported results is directed to the lead content of the Bones, the Lungs, the Liver and Spleen, and the Brain.
3. From this Analysis it is concluded that,
 - I. The Bones are the main site of stored lead in the body.
 - II. That the Short Bones by reason of their greater content of Red Marrow contain relatively more lead than do the Long Bones.
 - III. The low lead content of the Lungs is considered to be confirmatory of the earlier criticism of the theory that Lead Inhalation is the main source of absorbed lead.
 - IV. It is seen that the Liver of all viscera, has the heaviest lead concentration; this is considered to be due in part to lead liberated in the Spleen and

reaching the Liver by the Splenic Vein. The possible role of lead in the production of Cirrhosis of the Liver is also discussed.

- V. It is also concluded that the high lead content of the Liver is confirmatory of free Alimentary Absorption of Lead.
- VI. The low Lead Content of the Brain is concluded to indicate that lead is not normally deposited to any great extent in Nervous Tissues.
4. The use of Radio-Active Isotopes of Lead to determine lead distribution in the body is not considered to be of value.

SECTION IX. PART 2.

1. The previous methods of analysis for lead in cerebro-spinal fluid are reviewed.
2. The investigations of the lead content of the cerebro-spinal fluid are given along with the results obtained.
3. It is concluded from this investigation that:-
 - I. Lead is normally present in cerebro-spinal fluid.
 - II. The concentration is variable, and in some cases is greater than that of an equal volume of whole blood.
 - III. The ratio of blood lead to cerebro-spinal fluid lead varies from $1/3$ - $1/4$.
4. It is suggested that analysis of cerebro-spinal fluid lead should be undertaken in all cases of suspected Lead Encephalopathy.
5. The desirability of repeated estimation of the cerebro-spinal fluid lead where Deleading Measures are being undertaken is stressed.
6. It is not considered that in the nervous manifestations of lead poisoning that blood analyses alone is satisfactory.

SECTION X. PART 2.

1. The necessity of laboratory confirmation before a diagnosis of Lead Poisoning can be made is stressed.
2. The conflicting opinions of experts on the value of certain cardinal clinical signs and symptoms are discussed.
3. Attention is directed particularly to the factors which may contribute to an atypical Blood Picture in Lead Poisoning, and the unreliability of diagnostic significance being attached to the presence of Punctate Basophilia, and the Burtonian Line.
4. Stress is laid on the danger of diagnostic error when reliance is placed on the analysis of a single sample of blood.
5. The Normal Blood Lead Concentrations reported by different workers are given, and the variation in Blood/Urine Lead Ratios in health and in Lead Poisoning.
6. The necessity is stressed of analysis of the Cerebro-spinal Fluid where "Test" or Therapeutic Deleading is being carried out.
7. The necessity of Cerebro-spinal Fluid Analysis where Lead Encephalopathy is diagnosed is stressed.
8. The choice of Biological Material on which to carry out analysis is discussed.

9. The Diagnostic Criteria which I consider to be of value in the diagnosis of Lead Poisoning are listed.

SECTION XI. PART 2.

1. The Metals associated with Lead in Blood and biological material are tabulated, and discussed.
2. The possible function of the "Trace Elements" is discussed.
3. The effects of lead on the Experimental Animal, and the reproduction of experimental findings in clinical Lead Poisoning are discussed.
4. Attention is directed to the apparent Biochemical Relationship between Lead and Iron.
5. The question as to whether lead has a Physiological Function is discussed.

Note on the quotation from the Epidemia.

Ramazzini in this reference to Hippocrates gives the text as Book 4, section 13. Unfortunately as far as I can discover only Books 1 and 3 are now extant. In Book 3, section 34, there is a passage very similar of context, and it is not unlikely that in its passage through the hands of different translators that both the text and references have become corrupt.

The Latin is obviously impure. The use of Spirituosus which I take literally to mean 'full of breath' or 'wheezy' is not found in classical Latin dictionaries. Ramazzini's translator renders this word as 'Asthmatick', and in view of the expiratory difficulty in asthma deserves congratulation on his rendering. The punctuation of the original text is faulty; in my opinion 'Subdura' should go with 'alvus intenta' to give 'costive' literally a 'hard, strained, belly'. It is easy to translate this as not only a reference to lead constipation but also to lead colic - 'costive and colicky'.

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